Determination of acetate and formate in vegetable oils by ion chromatography after multivariate optimization of the extraction process using a Doehlert design

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ABSTRACT

In this work, we present the development of a method for the determination of acetate and formate in vegetable oils by ion chromatography with conductometric detection following their extraction from samples using a diluted KOH solution. The extraction procedure was optimized using a multivariate approach. The application of a 2^4 full factorial design showed that the mass of sample, extraction time, and KOH concentration presented significant influence on the extraction of both acetate and formate, whereas the temperature presented little influence on the process. As a result, we set the extraction temperature at 22 °C and performed the multivariate optimization of the other variables using a Doehlert design. The optimum conditions were: 4.8 g of sample, 8 mmol L^{-1} KOH solution and 19 min extraction time. Six samples of vegetable oils (soybean, corn, canola, sunflower and olive) were analyzed and recovery tests provided recovery percentages in the range of 82–118%.

1. Introduction

Vegetable oils are widely used in various types of industries. They are obtained from different seeds and fruits, such as olive, sunflower, corn, and soybean. The role of these oils in human nutrition is important because they allow the absorption of some vitamins and act as an energy source (Bastos, Costa, & Pereira, 2017; Trindade, Dantas, Lima, Ferreira, & Teixeira, 2015).

Vegetable oils are composed of triglycerides, which are glycerol molecules esterified with fatty acids. These oils can be degraded for distinct reasons, such as unsuitable packaging and storage conditions, but degradation can also be due to lipid oxidation as a result of heating. The presence of some chemical species, such as metals and phenolic compounds, can also accelerate the oxidative processes, decreasing the quality of the product. Among the substances formed in these oxidative processes, the small organic acids, particularly formic and acetic acid, are present (Kiritsakis, Kanavouras, & Kiritsakis, 2002; Souza, Ansolin, Batista, Meirelles, & Tubino, 2017). In this context, the identification of these acids is especially important because it can indicate the degree of degradation of the oil. The concentration of other substances, such as hydroxyperoxides, alcohols, and free fatty acids, can also be used for this purpose (Bastos et al., 2017; Rekas, Scibisz, Siger, & Wroniak, 2017; Vaskova & Buckova, 2015).

The determination of organic and inorganic substances present in vegetable oils is an analytical challenge because of the complex nature of the matrix, which is characterized by high carbon content. Some extraction methodologies have been proposed for the determination of metals (Leonardis, Macciola, & Felice, 2000; Ooms & Pee, 1983; Robaina, Brum, & Cassella, 2012) and inorganic anions (Dugo et al., 2007; Lemos, Cassella, & de Jesus, 2015) in edible oils. The determination of acetate and formate in biodiesel by ion chromatography with conductometric detection was proposed by Silveira, Caland, and Tubino (2014). In this case, the analytes were extracted from samples using an ultrasound-assisted method. However, none have been fully optimized, making the identification of the main parameters that influenced the extraction process impossible.

The term optimization is used in analytical chemistry to define a process that is performed to achieve the best response for a given system or method. Therefore, it can be said that the main goal of an optimization process is to improve the performance of a system (or a method) to obtain maximum benefit with minimum effort. The optimization process can be carried out through a univariate strategy, in which the influence of one factor is monitored at a time; or through a multivariate approach, in which several factors are evaluated simultaneously. Although the results obtained in a univariate optimization are easier to interpret, it requires larger amounts of reagents and longer

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times to be accomplished. On the other hand, multivariate approaches allow one to estimate the interactions among factors, yielding a more efficient optimization. Additionally, multivariate optimization tends to be more economical in comparison with a classic univariate approach since, in general, a lower number of experiments is required (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Ferreira, Bruns, da Silva & et al., 2007).

Multivariate optimization of a given system (or method) is generally performed in two steps. In the first step, the significance of the chosen factors (or variables) is evaluated, whereas the actual optimization is performed in the second step. The first step is commonly completed using screening designs, such as the full-factorial, fractional factorial, and Plackett–Burman designs, whereas the use of a response surface methodology is the most popular way to perform the second step. The most employed response surface designs include the Central Composite, Box–Behnken, and Doehlert designs (Ferreira, Bruns, Ferreira & et al., 2007; Ferreira, dos Santos, Quintella, Neto, & Bosque-Sendra, 2004; Novaes et al., 2016). The Doehlert design presents some advantages over the Central Composite and Box–Behnken designs, such as the highest efficiency and a lower number of experiments required to complete the system modeling (Bezerra et al., 2008; Ferreira et al., 2004). The Doehlert design was proposed in 1970 (Doehlert, 1970), but its first application in analytical chemistry only dates to 1989, when Hu and Massart (1989) tested this design to optimize the experimental conditions of different chromatographic separations. Since then, it has been used successfully in the optimization of various experimental procedures (Brum, Lima, Robaina, Fonseca, & Cassella, 2011; Caldas, de Paula, Brum, & Cassella, 2013; Caldas, Francisco, Pereira-Netto, & Cassella, 2011; El Ati-Hellal, Hellal, & Hedhili, 2014; Ellouze et al., 2017; Ferreira, Bezerra, dos Santos, & Neto, 2003; Robaina, Soriano, & Cassella, 2009).

The goal of this work was to perform a multivariate optimization of the extraction of acetate and formate from vegetable oils using a Doehlert design. The initial screening of the variables (mass of sample, extraction time, KOH concentration, and temperature) was carried out by a full factorial design in two levels. The determination of both acetate and formate in the extracts was performed by ion chromatography with conductometric detection.

2. Experimental

2.1. Apparatus

All determinations of anions in the aqueous solutions (sample extracts and standard solutions) were carried out with a Dionex Ion Chromatography System, model ICS-2100 (Sunnyvale, CA, USA), equipped with an integrated eluent (potassium hydroxide) generator, model RFIC-EG (EGC III KOH cartridge), and an AERS 500 2 mm membrane suppressor. Conductivity signals were measured with a DS6 heated conductivity cell and the chromatograms were acquired and registered using Chromeleon software, version 7.2, supplied by Dionex.

Separation of the anions was performed with an Ion-Pac AS11HC analytical column (2 × 250 mm, 13 μm particle size) and an IonPac AG11 guard column (2 × 50 mm, 13 μm particle size) to protect the analytical column. The eluent was pumped at a flow rate of 0.40 mL min^{-1} using a gradient program with a potassium hydroxide solution: 0–5 min (2 mmol L^{-1}); 5 to 10 min (33 mmol L^{-1}); and 10 to 18 min (2 mmol L^{-1}). The samples and standard solutions were injected using an autosampler and a 25-μL loop injector. Under these conditions, acetate and formate peaks appeared at 4.46 and 5.46 min, respectively. A current of 33 mA was applied to the suppressor device.

2.2. Reagents and solutions

The deionized water used in this work was obtained using a Direct Q3 water system from Millipore (Bedford, MA, USA), and always had a resistivity higher than 18.2 MΩ cm.

Aqueous stock solutions of acetate and formate were prepared from their respective sodium salts to give a final concentration of 1000 mg L^{-1}. All solutions employed in this work were prepared by a dilution of the stock solutions with deionized water.

Potassium hydroxide solutions were prepared by dissolving a suitable mass of high-purity KOH, supplied by Sigma-Aldrich (St. Louis, MO, USA), in deionized water.

2.3. Material decontamination

All flasks used in this work were decontaminated by soaking in a 10% v/v HNO$_3$ solution for at least 24 h. After this time had elapsed, the flasks were rinsed with deionized water, dried, and stored in a clean and dust-free location until use.

2.4. Vegetable oil samples

All vegetable oil samples analyzed in this work (corn, canola, sunflower, soybean, extra virgin olive, and olive) were purchased at a local supermarket in the city of Niterói, Rio Janeiro, Brazil. They were wrapped in aluminum foil for protection from light and stored in the dark at room temperature to reduce lipid oxidation.

2.5. Optimization strategy

The optimization strategy employed in this work was comprised of two steps. In the first step, we applied a two-level full factorial design for the identification of significant variables. The full-factorial design generated a matrix with 16 experiments, which were run in triplicate. The variables considered for optimization of the extraction method were: concentration of KOH in the extractant solution (0–10 mmol L^{-1}), extraction time (1–19 min), sample mass (2.0–8.0 g), and extraction temperature (22–55 °C). In all cases, the volume of extractant solution was constant (30 mL) and the extraction was performed by magnetic stirring (Fig. S1). Once the significant variables were identified, we applied a Doehlert design for optimization of their levels. A soybean oil was employed in all optimization experiments and the relative responses (in terms of peak area) for acetate and formate found in this sample were used as quantitative responses in data processing. The responses were normalized using the mass of sample weighted in each experiment. The data were processed using the software Statistica, version 7.

2.6. General sample extraction procedure

In every optimization experiment, a given mass of soybean oil was weighed in a clean 50 mL polyethylene tube and 30 mL of extractant solution was added. Then, the tube was capped, immersed in a waterbath with controlled temperature, and the mixture was stirred for different times, which were established according to the conditions indicated by the design. Subsequently, the tube containing the mixture was removed from the water-bath and centrifuged for 15 min at 5000 rpm to separate the organic and aqueous phases. The aqueous phase was collected, diluted with deionized water when necessary, and filtered through a polyvinylidene fluoride membrane with a 0.22 μm pore diameter. The final extract was injected into the chromatographic system for acetate and formate determination using analytical curves prepared with aqueous standard solutions of the analytes. The real samples were analyzed using the same procedure but employing the optimized conditions.

2.7. Analyte recovery assays

Recovery tests were performed to evaluate the accuracy of the optimized extraction method. In this procedure, the aqueous standard