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Article

Direct Determination of Oleic Acid in Soybean Oil by Capacitively Coupled Contactless Conductivity Detection Capillary Electrophoresis in an Oil-Miscible KOH/1-Propanol/Methanol Medium

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Este trabalho teve por objetivo desenvolver um método analítico direto e rápido para a determinação de ácido oleico em óleo de soja por eletroforese capilar com detecção condutométrica sem contato. O eletrólito de corrida empregado foi uma mistura metanol/1-propanol (1:6 v/v) contendo 4×10^{-2} mol L^{-1} de KOH e 10% (v/v) em etileno glicol. As amostras foram preparadas pela solubilização de 50 g L^{-1} de óleo de soja e $1{,}33 \times 10^{-3}$ de ácido salicílico (padrão interno) no eletrólito de corrida. Os ensaios quantitativos foram realizados adicionando ácido oleico puro às amostras, na faixa entre 0,53 e $2{,}13 \times 10^{-3}$ mol L^{-1}. Sob polaridade negativa, os solutos aniônicos deslocaram-se mais rapidamente do que o fluxo eletro-osmótico e o ácido oleico foi detectado em 16 minutos. Os limites de detecção e de quantificação foram, respectivamente, de 24 e 81 μmol L^{-1}. Tais resultados demonstram que baixos teores deste ácido graxo podem ser quantitativamente determinados no óleo de soja sem a necessidade de extração prévia, como requerem outros métodos.

The aim of this work was to develop a quick direct analytical technique for the determination of oleic acid content in soybean oil by non-aqueous capillary electrophoresis with capacitively coupled contactless conductivity detection. The oil-miscible background electrolyte was a mixture of methanol/1-propanol (1:6 v/v) containing 4×10^{-2} mol L^{-1} KOH and 10% (v/v) ethylene glycol. Samples of 50 g L^{-1} soybean oil were prepared directly in the background electrolyte added with 1.33×10^{-3} g L^{-1} of salicylic acid as internal standard. Quantitative tests were performed by adding to the samples pure oleic acid in the range from 0.53 to 2.13×10^{-3} mol L^{-1}. Under negative polarity anionic solutes moved faster than the electro-osmotic flow so that oleic acid was detected in 16 minutes. The limits of detection and quantification were, respectively, 24 and 81 μmol L^{-1}. Such results demonstrate that, unlike required by other methods, low levels of oleic acid can be quantitatively determined in soybean oil without prior extraction.

Keywords: oleic acid, non-aqueous capillary electrophoresis (NACE), contactless capacitively coupled contactless conductivity detection (C^4D), soybean oil

Introduction

It is well known that fats and oils decompose slowly during storage releasing their fatty acid (FA) constituents. Therefore, a quality and freshness assessment of fats and oils can be made based on the fatty acid content.

Currently, important interests in oleic acid determination are, among others, medical research, biotechnology and evaluation of the lipid distribution in foods.[1-4] In Brazil, since synthesis of biodiesel is heavily dependent on the production of soybean oil, quality control parameters, such as free fatty acid content in the feedstock, is imperative. Therefore, development of fast, reproducible and low cost methods for the determination of fatty acids is a target of great interest.

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The analysis of vegetable oils is generally preceded by extraction of an analyte using a suitable organic solvent.

The most commonly used method for analyzing fatty acids (FAs) involves the determination of their corresponding methyl esters using gas chromatography (GC). The lower temperatures required during the analysis to reduce the risk of isomerization of double bonds and the easy modification of retention characteristics by varying the mobile phase composition has increased the interest in using high-performance liquid chromatography (HPLC) as compared to GC, for which a derivatization procedure is mandatory to increase volatility and overcome adsorption of polar functional groups on the GC column.[5] However, since FAs are neither UV nor fluorescence-active, derivatization is usually performed to render them detectable through HPLC with UV-Vis detection. Nevertheless, problems in derivatization include longer analysis time and unselective labeling, leading to interfering by-products and unstable nature of some derivatization reagents. Thus, there has been a strong emphasis lately on developing alternative methods to traditional HPLC for the determination of underivatized FAs involving several detectors, including HPLC with evaporative light-scattering detection and capillary electrophoresis with capacitively coupled contactless conductivity detectors (C^4D).[6]

Capillary electrophoresis with C^4D is an alternative to conventional conductivity detection. The highest frequency of operation employed enables positioning the electrodes on the outside of the capillary, an arrangement that avoids contamination of the electrodes in addition to providing an uncoupling between the detection circuit and the high potential used in the separation.[7]

Sequential injection analysis (SIA) and flow injection analysis (FIA) methods have been developed for the determination of acidity in vegetable oils aiming to automate the analyses, thus enhancing laboratory productivity. Makahleh and Saad reported a FIA method for the rapid determination of free fatty acids (FFA) using C^4D.[8] However, a suspected excessive dispersion of the FA by the carrier stream led to the incorporation of a preconcentrator column between the injector and the detector.

Separation of linear chain fatty acids containing 8 to 20 carbon atoms by capillary electrophoresis with C^4D was first described in 2003. The method was successfully applied in the analysis of babassu coconut oil which was saponified prior to analysis.[9]

Recently, a capillary electrophoresis method with C^4D has been proposed for the analytical separation and simultaneous determination of eleven FAs, from C12:0 to C20:0. However, the proposed methodology requires a transesterification step of FA standards.[10]

In our previous non-aqueous capillary electrophoresis (NACE) study with UV detection, an oil-miscible KOH/1-propanol/methanol medium was employed to detect carboxylic acids and phenols.[11] The alkanol medium reverses the electro osmotic flow under negative polarity (anode at the injection end of the capillary) while the presence of KOH increases conductivity of the medium.

The present study aims to evidence the advantages of associating NACE and capacitively coupled contactless conductivity detectors in the direct quantification of oleic acid, a monounsaturated fatty acid ($C_{18}H_{34}O_2$), in soybean oil. The procedure renders any kind of pretreatment of the sample or of the standard fatty acid unnecessary, and uses small volumes of solvents.

Experimental

The following analytical grade solvents and reagents were used: methanol, ethanol, 1-propanol, NaOH and KOH (Synth, 97%), salicylic acid (Merck, PA), ethylene glycol (EG, 1,2-ethanediol) and oleic acid (Reagen, Brazil), distilled and deionized water. Refined and deodorized soybean oil stored in metallic containers was obtained from the local market.

Fused-silica capillaries of 45 cm (35.5 cm effective length) × 50 μm internal diameter (i.d.) and 150 μm outer diameter (o.d.) were used. New capillaries were treated with 0.1 mol L^{-1} NaOH (5 min), distilled and deionized water (5 min) and background electrolyte (BGE, 5 min), in this sequence. The capillary was rinsed daily and between consecutive runs with a mixture of 10% water in ethanol, v/v, containing 4% KOH. These procedures were carried out under negative pressure (–450 mm Hg) applied at the capillary outlet. After each working session, it was flushed with water for 10 min.

The background electrolyte was prepared with methanol/1-propanol 1:6, v/v, containing 4×10^{-2} mol L^{-1} KOH and 10% (v/v) ethylene glycol, the role of which was to extend the stability of the solution samples which, in its absence, showed a slight turbidity after 3 hours.

The separations were performed under –25 kV, using a home-made equipment provided with a capacitively coupled contactless conductivity detection with 0.5% variation in migration times.[12] The frequency of the oscillator was adjusted at 550 kHz with 8 V amplitude. The signal from the receiver electrode was rectified and amplified by components of the electrophoresis equipment and acquired on A/D mode of a potentiostat Autolab from Eco Chemie B.V. through programs for acquisition and processing of data 4.8 General Purpose Electrochemical

Table 1. Properties of MeOH, PrOH and EG at 25 ºC

Solvent	t_{boil} / ºC	η / (mPa s)	ε	p*K*	κ / (nS cm^{-1})
Methanol (MeOH)	64.7[13]	0.545[13]	32.70[13]	17.20[13]	1207[14]
1-Propanol (PrOH)	97.2[13]	1.956[13]	20.33[13]	19.43[13]	353[14]
1,2-Ethanediol (EG)	197.2[15]	16.1[15]	37.7[13]	15.84[16]	1070[15]

t_{boil}: boiling point; η: coefficient of viscosity; ε: relative permittivity; p*K*: autoprotolysis constant; κ: electrical conductivity.

System (GPES) from Eco Chemie B.V. and Microcal® Origin 8.0 from Microcal Software.

Quantitative studies were carried out in triplicate, using salicylic acid as internal standard and the separations were performed at 25 ºC. Before hydrodynamic injection (10 cm × 30 s) all solutions were filtered through 0.45 µm pore size polytetrafluoroethylene (PTFE, CHROMAFIL Xtra) membranes.

Equal aliquots of the background electrolyte containing 50 g L^{-1} soybean oil and 1.33 × 10^{-3} mol L^{-1} salicylic acid were spiked with increasing volumes of 5.7 × 10^{-2} mol L^{-1} oleic acid in methanol so that their concentrations in the standard addition curve were 0.53; 1.07; 1.60 and 2.13 × 10^{-3} mol L^{-1}.

Results and Discussion

Selection of the solvent mixture

In a previous work[11] it was observed that soybean oil is miscible with several polar solvents, at least in a 1:1 v/v ratio. In the context of NACE, 1-propanol (PrOH) presents an excellent balance of the required solvent characteristics (Table 1), e.g., high boiling point, low viscosity, high relative permittivity, low electrical conductivity in the presence of moderately concentrated buffers, and large thermal conductivity. Moreover, the properties of PrOH can be further improved, by mixing with moderate amounts of MeOH (observed in preliminary tests), since the latter shows lower viscosity and higher electrical conductivity.

Low viscosity and high relative permittivity solvents are essential to keep the ions separated. On the other hand, large thermal conductivity and low electrical conductivity are desirable in the presence of moderately concentrated buffers to avoid loss of efficiency due to the Joule effect.

The solvent mixture became both conductive and strongly alkaline by adding KOH.[11] Due to the acid character of all primary alcohols very high pH* values (pH* = pH in non aqueous medium) were expected to be necessary to ionize oleic acid in MeOH/PrOH mixtures. On the other hand, besides stabilizing samples for longer time, the presence of EG increases thermal conductivity without compromising the conductivity of the solvent mixture as required for electrophoretic separations.

Separation of oleic acid

Increasing oleic acid concentration in spiked samples changes the medium physical chemical parameters, such as viscosity, conductivity and permissivity so that a non-linear detector response behavior in relation to the variation in oleic acid content is to be expected. To surmount this limitation, an internal standard was employed.

Moreover, internal standard substances allow correcting variations of injected volume for both chromatographic and electrophoretic methods. They should, ideally, be chemically similar to and non-reactive with the substance to be measured or with other matrix components and present distinct retention times, distant from all other substances in the sample, yet close to that of the analyte.[17,18] Preliminary experiments showed that salicylic acid fitted these requirements.

The separation between salicylic and oleic acid peaks is evidenced in the electropherogram shown in Figure 1, with migration times of 13 and 16 minutes, respectively. Being the smaller monovalent anion in the sample plug salicylate migrates first, 3 minutes before the bigger one,

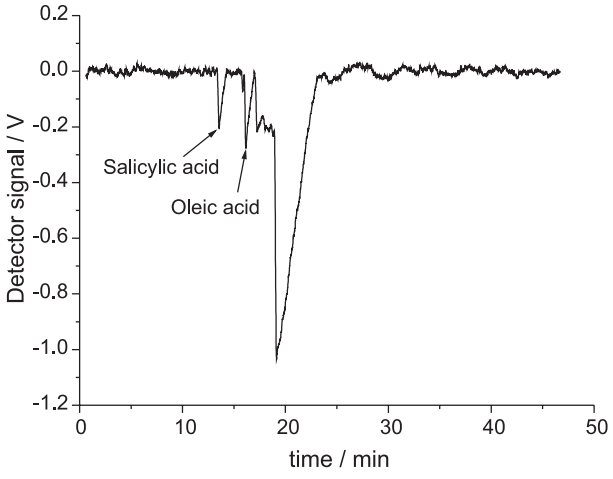

Figure 1. Electropherogram for 5.3 × 10^{-4} mol L^{-1} oleic acid added to the background electrolyte containing 50 g L^{-1} soybean oil and 1.33 × 10^{-3} mol L^{-1} salicylic acid as internal standard.

oleate. The presence of other species in the same migration times was discarded by injecting a non spiked sample. The high intensity large peak at 19 minutes corresponds to non-resolved components in the vegetable oil sample plug.

Possibility of formation of fatty acid alkaline salts by action of KOH on triacylglycerols was investigated starting from saponification tests. Measurements were performed in three steps, immediately after BGE preparation and after 3 and 6 h. The observed saponification index increase with time was small (4.3 to 11 mg KOH g^{-1}) as compared to that expected for soybean oil (189-195 mg KOH g^{-1})[19] suggesting that the peak at 19 min corresponds mostly to triacylglycerols.

Standard addition curve

Similar detector response behavior was obtained for oleic acid and salicylic acid peaks in the BGE containing 50 g L^{-1} of soybean oil sample and 1.33 × 10^{-3} mol L^{-1} of internal standard. Figure 2 shows salicylic (S_I) and oleic (S_O) acids mean peak areas from triplicate results for the blank sample and for those spiked with 5.3, 10.7, 16.0 and 21.3 × 10^{-4} mol L^{-1} pure oleic acid, respectively.

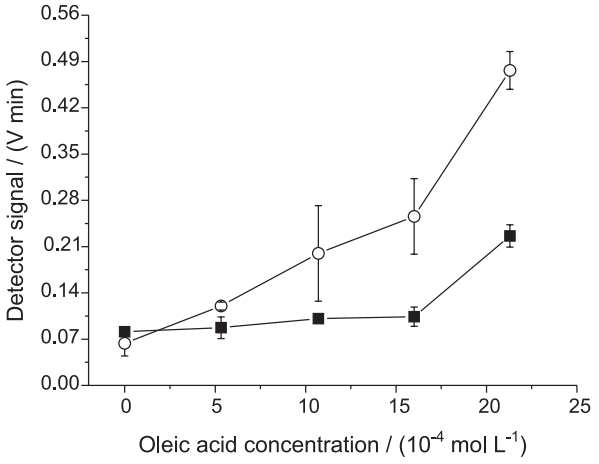

Figure 2. Oleic acid (○) and salicylic acid (■) peak areas, S_I and S_O, respectively, in background electrolyte containing 50 g L^{-1} soybean oil sample spiked with 5.3, 10.7, 16.0 and 21.3 × 10^{-4} mol L^{-1} pure oleic acid.

The variation of the detector response for salicylic acid is a result of increasing concentrations of oleic acid in the solution, which decreases the medium conductivity and increases its electrical resistance. However, despite the significant dispersion in the detector response observed for the higher concentrations, a linear relationship was obtained in the concentration range between 10^{-4} and 10^{-3} mol L^{-1} of p.a. oleic acid added to the soybean oil sample by dividing the signal area values generated for both salicylic (S_I) and oleic (S_O) acids, the correlation coefficient being $r = 0.999$ (see Figure 3).

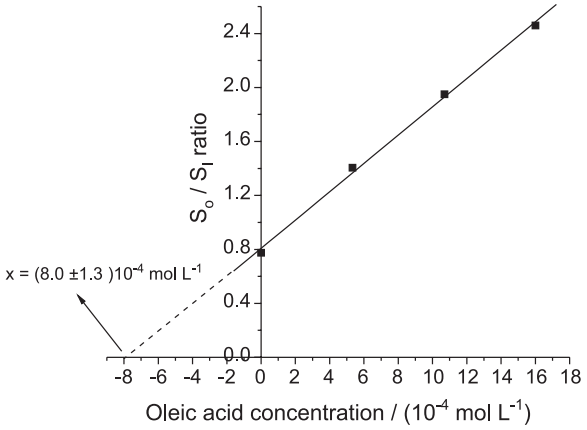

Figure 3. Standard addition curve for: blank, 5.3, 10.7 and 16.0 × 10^{-4} mol L^{-1} oleic acid in the BGE containing 50 g L^{-1} soybean oil and 1.33 × 10^{-3} mol L^{-1} salicylic acid.

The amount of oleic acid found in 100 g of sample was 0.4 ± 0.1 g, which is around 25% above data reported in the literature[19] for free oleic acid concentration in soybean oil and 33% above the Brazilian National Agency for Sanitary Surveillance (ANVISA)[20] specification, which is 0.3 g oleic acid *per* 100 g of oil. The calculated limits of detection and quantification were, respectively 24 × 10^{-6} and 81 × 10^{-6} mol L^{-1}, as shown in Table 2.

A sensitivity of 1055 mol^{-1} L calculated from the standard addition curve evidences that a low content of this fatty acid can be quantitatively determined in soybean oil, without previous extraction, through capillary electrophoresis employing C^4D.

Conclusions

Associating NACE and C^4D allowed quantification of oleic acid without any sample pretreatment using the standard addition method. A direct injection procedure resulted in a reproducible, low volume of solvent consumption, low migration time oleic acid determination

Table 2. Figures of merit

LOD[a] / (μmol L^{-1})	LOQ[b] / (μmol L^{-1})	Sensitivity / (mol^{-1} L)	Linear range / (mol L^{-1})	*r*
24	81	1055	up to 16.0 × 10^{-4}	0.999

[a]For signal to noise ratio (SNR) = 3; [b]for SNR = 10. *r*: correlation coefficient.

in soybean oil sample with high sensitivity. By using salicylic acid as internal standard, the proposed technique allows quantifying the analyte for characterization and/or fraud detection purposes. In comparison with direct injection in a miscible BGE, previous extraction has the advantage of preconcentrating the solutes but additional manipulation of the sample increases time of analysis and the risk of sample contamination as well as alteration on analyte concentration.

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