

Double use of microwaves in fatty acid preparation for elaidic acid determination as phenacyl ester using high-performance liquid chromatography in Brazilian fat products

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Abstract

Brazilian samples of fat products like margarines, butters and chocolates were prepared to reversed-phase high-performance liquid chromatography analysis, using a domestic microwave oven for fat hydrolysis and derivatization with phenacyl chloride. The total reaction time was 6 min, recoveries were above 94%. Total reproducibility for whole procedure were 3.5% R.S.D. for elaidic acid ($n=3$), the total chromatography analysis was 25 min.

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1. Introduction

Elaidic acid is the most common *trans* fatty acid (*t*FA) isomer [1,2], in reported *trans* fat tables [1–4] it is responsible for 80–100% of total *t*FAs. So, the determination of elaidic acid could be regarded as an important tool to assess the quality of food products regarding the *t*FA content.

Many methods have been proposed for its determination, especially by gas chromatography (GC) [5,6], which also requires derivatization. However, high-performance liquid chromatography (HPLC) derivatization methods are still based on long reaction times and some time-consuming routines, as purification of derivatives by solid-phase extraction (SPE) [7].

The goal of this study is to optimize conditions and develop a fast HPLC derivatization procedure equivalent to the existent GC ones, with the advantage that the shorter heating time could lead less to isomerization of fatty acids, for this, microwave ovens have been used for accelerating

sample pre-treatment as in transmethylation [8] and saponification [9].

2. Experimental

2.1. Reagents and samples

Phenacyl chloride was purchased from Aldrich (Steinheim, Germany), all solvents were from Vetec (São Paulo, Brazil), standard reference fatty acids and methyl esters were from Merck (Darmstadt, Germany).

Two margarine brands, one butter, one soy oil, and one chocolate bar sample were bought at local market to be used as reference matrices.

2.2. Instrumental

The column was an HP Hypersil (Hewlett-Packard, USA) 100 mm × 2.1 i.d., with ODS stationary phase, 5 μm particle size. The HPLC system was constituted by a LC-10 AD pump and SPD-10 UV-vis detector (Shimadzu, Japan), set up to 245 nm, all elutions were isocratic runs at 400 μl/min (85:15

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methanol:water) at room temperature. The volume injected was 20 μ l.

2.3. Reaction conditions

Reactions were carried out in a domestic microwave oven at 160 W (20% of total exit power), using closed polypropylene vials.

For saponification, 50 μ l of 20 mg/ml samples and 50 μ l of C17:0 (margaric acid) 1 mg/ml (internal standard), both in toluene, were placed in a polypropylene vial with 50 μ l of KOH 2% in ethanol and reacted for 3 min; after that, 100 μ l of acetic acid 10% in water and 100 μ l of toluene were added and vortex mixed for 15 s; after phase separation, 50 μ l of organic phase was withdrawn to other vial and dried at stream of nitrogen at room temperature. For phenacyl derivatization,

the procedure of Wood and Lee [10] was adapted. Briefly, to the dry free acid residue, 100 μ l of phenacyl chloride and triethylamine (both 20 mg/ml in acetone) was added, vortex mixed for 15 s and reacted for 2 min. The next step was to destroy the excess of reagent, using 50 μ l of acetic acid, and again reacting the vial at microwave oven for 1 min. One hundred and fifty microliters mobile phase was added to the vial, for dilution and injection.

3. Results and discussion

Resolution of C14 (myristic) and C18:3 (linolenic) acid failed with methanol–water mobile phase, as shown in Fig. 1, the critical pair of palmitic (16:0) and oleic (18:1) acids were well resolved as C18:1 isomer, elaidic acid.

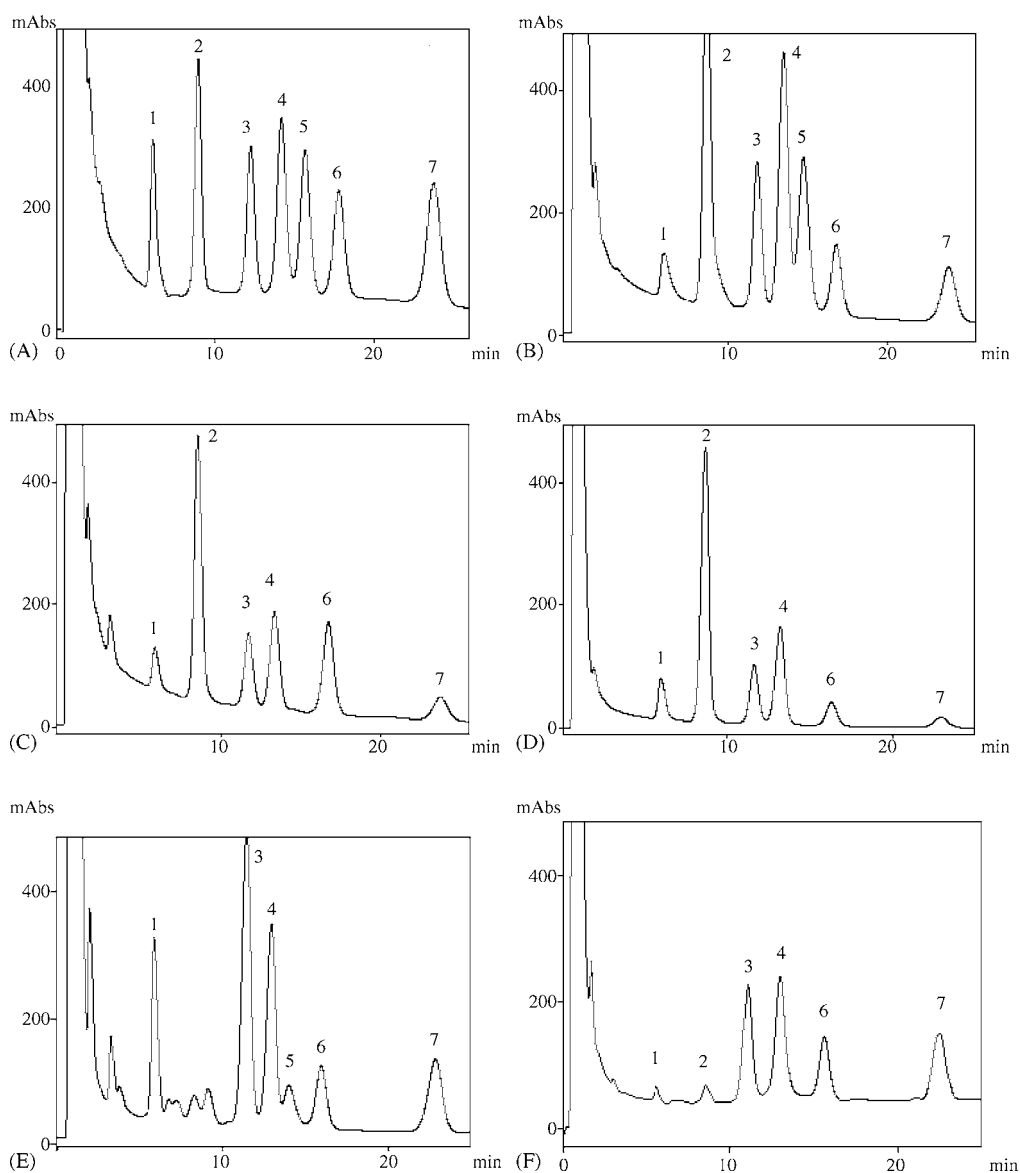


Fig. 1. RP-HPLC of: A, standard mixture; B, margarine brand A; C, margarine brand B; D, soy oil; E, butter and F, chocolate. For HPLC conditions see text. Peaks are: 1, linoleic + myristic; 2, linoleic; 3, palmitic; 4, oleic; 5, elaidic; 6, margaric (internal standard); 7, stearic phenacyl ester.

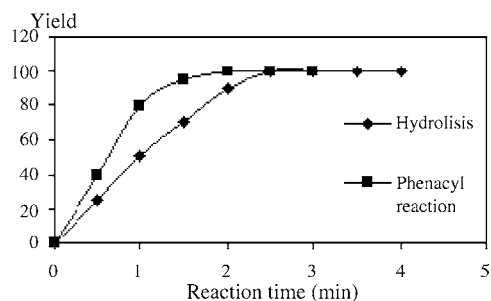


Fig. 2. Effect of irradiation time in hydrolysis and esterification of elaidic acid for HPLC analysis.

No attention was taken in the resolution of C18:3 (linolenic) and C18:2 (linoleic) *trans* isomers, that can be achieved by reversed-phase (RP) HPLC [10,11] or by silver-ion techniques [12].

It is worthy to note that RP resolves only geometrical isomers, double bond positional isomers can be separated more efficiently by GC with high polar phases [13].

Microwave saponification is delicate to perform especially because of rapid heating and overpressure problems with ethanolic solutions [9], an interesting solution was found by Jeyashoke et al. [8] that used mixtures of toluene and methanol in transmethylation reactions to decrease the fast heating and overpressure of reaction solutions. Working with toluene mixtures and small volumes (150 μ l), up to 15 vials could be reacted simultaneously for hydrolysis without lost in yield or any safety risk.

The completeness of the reactions were calculated using different times for hydrolysis and only one to phenacyl reaction, and then, with optimal hydrolysis time, different reactions times were tried for phenacyl derivatization. This approach is schemed in Fig. 2.

The chocolate sample, although the already expected composition of C18:0 12%, C18:1 12% and C16 11%, showed a low level of myristic acid that was expected since milk fat is also present.

One of the margarines (margarine brand B) did not present any elaidic acid. This fact was not expected, since hydrogenation process lead to isomerization. The manufacturer claim that it uses an exclusive process, that we believe to be transesterification [14] using some short chain saturated oil like palm or coconut oil [15].

The quantitative data of all fatty acids in these samples are in agreement with published composition data [16]. Only one margarine and a butter sample presented elaidic acid content, they were 16.6 and 4.8% (w/w) respectively.

Reproducibility of whole procedure (hydrolysis and phenacyl derivatization) was 3.4% ($n = 3$). The detection limit ($S/N = 3$) was 30 ng or 0.25% of standard starting amount of 20 mg/ml.

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