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Food Chemistry 88 (2004) 609–612

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Microwave-assisted preparation of sugars and organic acids for simultaneous determination in citric fruits by gas chromatography

Fabiano O. Silva *, Vany Ferraz

Departamento de Química, Universidade Federal de Minas Gerais, Av. Antonio Carlos 6627, Belo Horizonte-MG, 31270-901, Brazil

Received 2 October 2003; received in revised form 10 May 2004; accepted 11 May 2004

Abstract

A simple and rapid analysis of sugars and organic acids by gas chromatography (GC) has been developed. Fructose, glucose, sucrose, citric and malic acid in citric fruits were quantitatively derivatized in two reactions. The total derivatization time was 5 min using a domestic microwave oven at 180 W radiation power. Average analytical recoveries were above 97%. The method demonstrate be 20 times faster than others found in the literature and ideal for routine analysis.

Keywords: Gas chromatography; Sugars; Acids; Trimethylsilyl; Oxime; Microwave assisted

1. Introduction

Sugars and acids are well established as chemical markers in quality, ripeness, authenticity and as distinguishable feature of food origin. Brazil is one of the greatest worldwide producer of citrus and provides more than 80% of the international frozen concentrate orange juice market (FAO, 2002), for such importance, reliable, fast and low-cost analytical methods are needed for assure the above cited features.

Chromatographic analysis of sugar and acids have been troublesome for a long time, sugars are naturally non-volatile, lack of UV absorbing moieties and the main chromatographic technique is the high performance liquid chromatography (HPLC) with NH2 bonded-phase and refraction index detection (Li, Andrews, & Pehrsson, 2002; Nollet, 1992). Gas chromatography (GC) faced problems of multiple peaks due to the anomers of cyclic forms (Martinez-Castro et al., 1987) and long reactions times and multiple steps sample preparation (Bradbury, Halliday, & Medcalf, 1981; Molnár-Perl & Morvai, 1990; Molnár-Perl, Morvai, & Knausz, 1991). The multiple peak problem were solved by reacting the C1 position of the acyclic form by reduction to alditols (Bradbury et al., 1981) or by means of oxime formation with hydroxylamine (Molnár-Perl & Morvai, 1990, 1992; Molnár-Perl et al., 1991 ; Molnar-Perl, Katona, & Sass, 1999) as in the early studies of Sweeley, Bentley, Makita, and Wells (1963) prior to the derivatization step. Reduction to alditols have the advantage that each sugar generate only one peak, while trimethylsilyl (TMS) oximes give 2 peaks, but hydroxylamine reactions are more selective. Alditols preparation also include cation-exchange steps and boric acid removal (Bradbury et al., 1981), in the TMS-oxime preparation, all reactions are carried out in the same vial and consecutively.

To shorten the sample preparation process, microwave radiation offers a good way for supplying high acceleration rates in a short time for organic reactions (Caddick, 1995). Further discussions about chromatographic techniques in sugar and organic acids analysis are available (Molnar-Perl, 1999, 2000).

The aim of this study is to optimize the reactions conditions for large number of samples and routine analysis by GC. This study was applied to five target compounds (malic acid, citric acid, fructose, glucose, sucrose) in Brazilian varieties of oranges and lemons as model matrices.

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* Corresponding author. Fax: +55-31-3499-5700.
E-mail address: drhtcl@yahoo.com (F.O. Silva).

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doi:10.1016/j.foodchem.2004.05.002

2. Experimental

2.1. Materials

All authentic sugars and acids, as well as hydroxylamine and hexamethyldisilazane (HMDS) were purchased from Sigma (Bellefont, PA, USA), pyridine of analytical grade were from Nuclear (São Paulo, Brazil).

2.2. Instrumental and analysis conditions

The gas chromatography (GC) were performed in a Varian CP-3380 (Walnut Creek, CA, USA). The column used was from Chrompack (Middelburg, Netherlands) (CP-Sil-5, 15 m × 0.32 mm I.D., 0.25 μm film thickness) temperature was: 100 °C for 1 min and then 300 °C at 20 °C/min rate. The flame ionization detector (FID) were at 300 °C and injector port also in 300 °C, 1 μl were injected using a split ratio of 1:100.

The freeze dryer equipment was an Edwards (West Sussex, UK) Modulyo with a vacuum chamber of 0.1 mBar and a −60 °C trap coil.

The derivatization reactions were performed in a domestic microwave oven with multimode irradiation set at 180 W, 30% of exit power. The reactions occurred in closed polypropylene vials.

2.3. Sample preparation and reaction conditions

Oranges of “Pera Rio” variety and lemons of “Tahiti” variety were purchased in local market, hand squeezed, centrifuged at 10,000 rpm by 4 min, filtered through 0.45 μm membrane filter.

The oxime-TMS formation was done as follow: 100 μl of the filtrate or standard stock solution of 1mg/ml of each sugar or acid was freezed inside the vial with liquid nitrogen then lyophilized. After this, 100 μl of 2% hydroxylamine in Pyridine was added to the vial, closed and introduced to the microwave oven for reaction by 1 min. The trimethylsilyl (TMS) reaction was accomplished with 500 μl of HMDS/pyridine (2:8) with microwave radiation for 4 min, if dilution was required, pyridine was used as the solvent. Finally 1 μl was injected in the chromatograph.

3. Results and discussion

Although the reaction rate could be increased using microwave radiation, a time-consuming drying step is still necessary for the silylation. Lyophilization shows to be less aggressive to thermal-labile compounds and more fast and easy to automate then drying with other methods such rotary evaporator when dealing with large sample number or routine analysis.

Domestic microwave ovens have been widely used in sample preparation, such as digestion (Smith & Argenault, 1996), hydrolysis (Piñero-Avila, Amparo, & Guardia, 1998) or esterification (Jeyashoke, Krisnangkura, & Chen, 1998), with or without modifications. When using commercial ovens, the major concern is the choice of the correct vessel that withstand the pressure. When high-pressure is required sealed glass vessels can be used surrounded by Vermiculite (Caddick, 1995). The source of microwave acceleration include the thermal effect associated with the dielectric polarization phenomenon of the alignment to the electrical field and a supposed athermal effect that is still ambiguous (Larhed & Hallberg, 2001; Smith & Argenault, 1996). This effect rely on the fact that the energy transmitted by microwave is too small (<0.3 Kcal mol^{-1}) to excite any molecular activation and others factors as kinetics must be regarded. Although the reaction rates could be accelerated in this study for the trimethylsilylation comparing to the direct heating method, the temperature in the microwave reaction mixture was lower than 80 °C in the end of 4 min while the classical method range from 20 min to 1 h (Bartolozzi, Bertazza, Bassi, & Cristoferi, 1997; Chapman & Horvat, 1989; Molnár-Perl et al., 1991, 1999; Molnár-Perl & Morvai, 1990, 1992; Streeter & Strimbu, 1998) reaction time in 70–100 °C heating. The acceleration of the mutorotation of the hemiacetal to the acyclic aldose/ketose under microwave irradiation was not found reported in the literature.

Since the derivatization of sugar include two different reactions, a careful control of conditions is necessary.

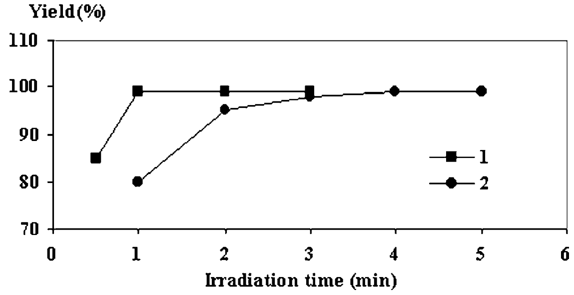

Fig. 1. Effect of irradiation time on the yield of glucose at 180 W power; 1 = oxime formation, 2 = trimethylsilylation.

Table 1
Recoveries (%) results of sugars/acids in different steps of preparation of orange juice for GC analysis

Compound	Conditions	
	Filtration/centrifugation	Microwave reactions
Citric acid	98	98
Malic acid	99	98
Glucose	97	95
Fructose	96	97
Sucrose	98	100

First the completeness of the oxime reactions was calculated using “standard” conditions for silylation (8 min at 180 W). After proof that the reaction time converts all sugars to their oxime forms, the next step was to find the optimal (shorter) reaction time for the TMS derivative. The completeness of the reaction was calculated plotting the ratio of peak areas to the reaction time in both cases. During the reaction period studied, no destruction of the already reacted compounds were noted after final reaction time, however, high powers at the same reaction time, decreased the recovery probably due to overheating and destruction of some sugars giving side-products in the chromatograms.

Fig. 1 shows the results of the oxime and TMS formation for glucose at different reaction times. Hydroxylamine reactions were already reported in water under microwave irradiation (Strassnig, Wenzl, & Lankmayr, 2000) these initial conditions guide us to find the optimal time.

To avoid losses in the sample pre-treatments steps, the samples were spiked with a known amount of compounds, about 50% and 100% of the literature value, not consecutively before the centrifugation and microwave reactions. Table 1 summarize the results of recovery in different sample preparation steps for the five target compounds in an orange sample. The total recovery value was 97.6% and the RSD 3.2% calculated from triplicate assays.

As we can observe in Fig. 2, the chromatographic analysis can be accomplished in less than 15 min. No loss in resolution between critical pairs, as in the two oxime TMS peaks (syn and anti position) of fructose and glucose, was noted as is showed in Fig. 3. The ratio of the isomers were constant, 1:1 for fructose and 4:1 for glucose as in Chapman and Horvat, 1989, and the peak areas were summed for quantitation purposes.

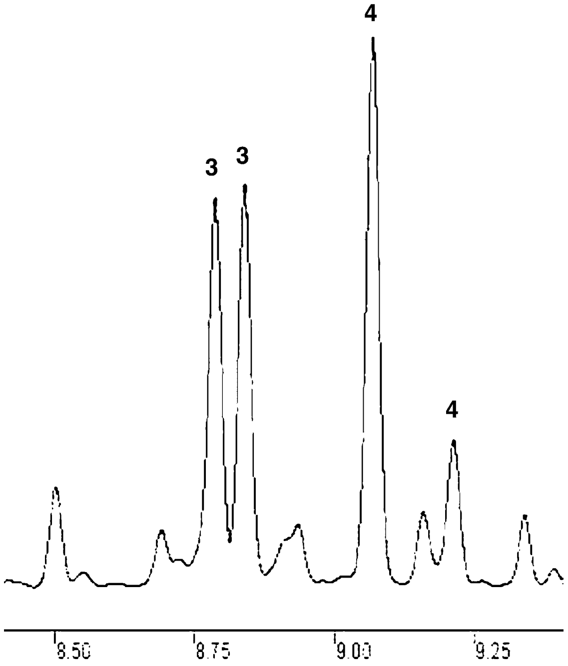

Fig. 3. Detail in the region of fructose and glucose in a lemon sample. Peaks: same as Fig. 2.

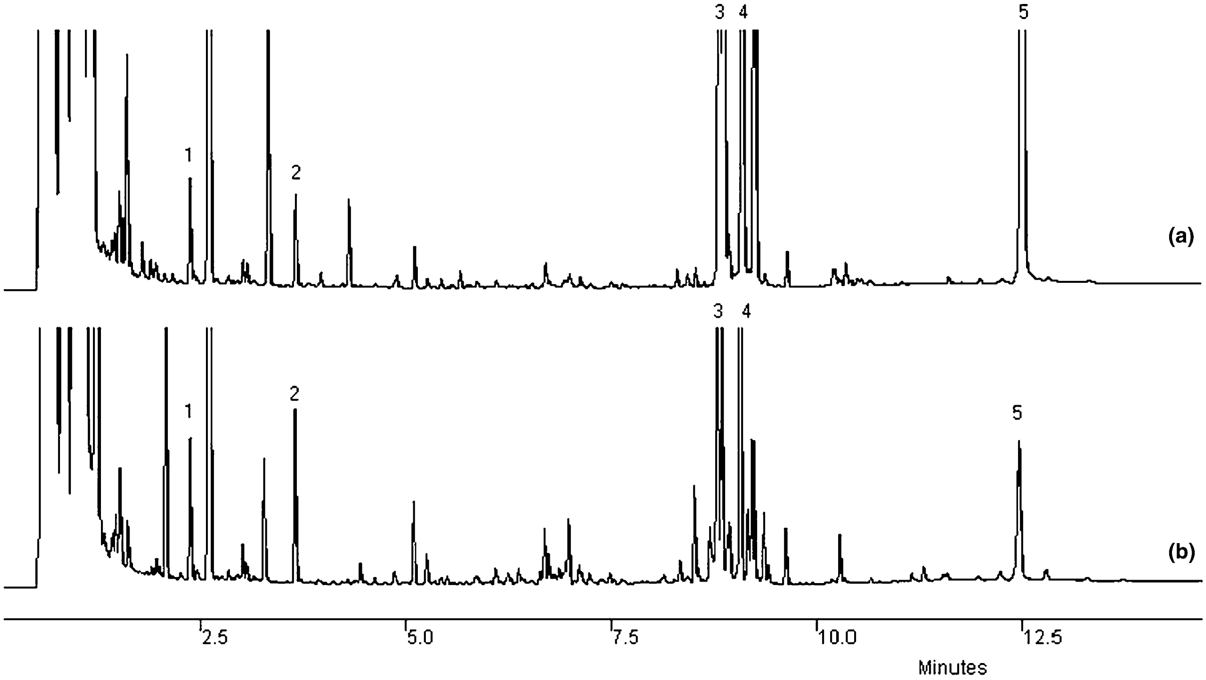

Fig. 2. Chromatogams of: (a) an orange sample, (b) a lemon sample. Peaks are: 1 = malic acid, 2 = citric acid, 3 = fructose, 4 = glucose, 5 = sucrose.

No specific data was found about sugars and organic acids composition in these Brazilian varieties, however, the sugar content is in agreement with general reported tables of others citrus varieties (Molnar-Perl, Morvai, Pintér-Szakács, & Petró-Turza, 1990; Monár-Perl & Morvai, 1992; U.S. Department of Agriculture, 1987).

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