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DETERMINATION OF CAPSAICINOIDS FROM DRIED PEPPER FRUITS BY FAST-GAS CHROMATOGRAPHY

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ABSTRACT

The present paper describes the application of Fast-gas chromatography (GC) to quantify the capsaicinoid content in dried pepper fruit with the aim to calculate the Scoville heat values (SHVs) for routine quality control. Fast-GC was used to analyse various samples of dried pepper fruits with a wide distribution of SHVs. The calibration curves demonstrated a polynomial model in the range of 3.0-195.0 $\mu\text{g mL}^{-1}$ for capsaicin and 2.6-105.0 $\mu\text{g mL}^{-1}$ for dihydrocapsaicin and nordihydrocapsaicin, with correlation coefficients of 0.996 and 0.997, respectively. The data showed an intraday relative standard deviation (RSD) <5% and an interday relative repeatability standard deviation (RSDr) <6%.

- Key words: *Capsicum*, spice, fast-gas chromatography -

INTRODUCTION

Pepper fruits (*Capsicum* spp.) are among the most commonly consumed spices having significant pungency. They contain capsaicinoids, derivatives of phenylpropanoid compounds, which are largely responsible for the hot taste, pungency, and trade quality. Eleven capsaicinoids have been identified, including amide derivatives of vanillylamine and carboxylic acids with eight to eleven carbon atoms, some unsaturated (GARCÉS-CLAVER *et al.*, 2006; KOZUKUE *et al.*, 2005). The capsaicinoid content can be inferred by the heat value based on the Scoville organoleptic test, which was developed by the American Spice Trade Association (ASTA). However, the Scoville Heat Value (SHV) is not always reliable and various authors have attempted to replace this method with more accurate instrumental measurements of the capsaicinoid content. When evaluating pungency, capsaicin (*trans*-8-methyl-N vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide) constitute 90% or more of the total capsaicinoids, and they account for the majority of the "hotness" (LASKARIDOU-MONNERVILLE, 1999). Nevertheless, the AOAC Official Method 995.03 (AOAC, 1995) recommends calculating the total capsaicinoid content as the sum of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin; then, the Scoville Heat Unit (SHU) for a given compound is determined by multiplying the total content of these capsaicinoids (expressed as $\mu\text{g g}^{-1}$ capsicum) by the pungency value for "pure capsaicin" (15 SHU). The SHV of the product is equal to the sum of the single SHU values. TODD *et al.* (1977) showed the capsaicinoid content could be converted to a more accurate SHU value by multiplying the content of each capsaicinoid by a coefficient: 16.1 for both capsaicin and dihydrocapsaicin, 9.3 for nordihydrocapsaicin, and lower coefficients for other capsaicinoids. The lowest coefficient was 6.9 for homocapsaicin.

Several varieties of *Capsicum* (Solanaceae family) have been distinguished and described; five are commonly recognised as domesticated: *Capsicum annuum* L. var. *Annuum*, *Capsicum baccatum* L. var. *Pendulum*, *Capsicum pubescens*, *Capsicum frutescens* L. (Cayenne pepper), and *Capsicum chinense* (PETER, 2001). Many different techniques are used to extract capsaicinoids from dried plant matrices, such as maceration (KIRSCHBAUM-TITZE *et al.*, 2002), magnetic stirring (CONTRERAS-PADILLA and YAHIA, 1998), enzymatic extraction (SANTAMARÍA *et al.*, 2000), a solvent and shake-flask extraction (Soxhlet) (KOREL *et al.*, 2002; KRAJEWSKA and POWERS, 1987), extraction by supercritical fluids (DAOOD *et al.*, 2002), extraction by pressurized liquids (BARBERO *et al.*, 2006a), and ultrasound assisted or microwave assisted extraction (BARBERO *et al.*, 2006a; BARBERO *et al.*, 2008a; KARNKA *et*

al., 2002; NAZARI *et al.*, 2007; WILLIAMS *et al.*, 2004). In general, there is always demand for simple extraction techniques, reduced organic solvent consumption, and low toxicity solvents. In particular, the AOAC Official Method 995.03 (AOAC, 1995) suggests extraction with warm ethanol in a reflux condenser, liquid chromatography, and fluorimetric quantification. COLLINS *et al.* (1995) also suggested extraction with acetonitrile at reflux and high pressure liquid chromatography (HPLC) for quantification, but BARBERO *et al.* (2006b) studied the extraction of capsaicinoids with various solvents and found acetonitrile is a fairly efficacious solvent, but less so than ethanol and methanol.

In recent years, reverse phase HPLC was the most frequently used method for quantification of capsaicinoids (BARBERO *et al.*, 2008b; CHOI *et al.*, 2006; KAALE *et al.*, 2002; MAILLARD *et al.*, 1997; PERUCKA and OLESZEK, 2000; SARIA *et al.*, 1981; THOMPSON *et al.*, 2005; WEAVER and AWDE, 1986). HPLC followed by mass spectrometry (HPLC/MS) was proposed more recently (GARCÉS-CLAVER *et al.*, 2006; KOZUKUE *et al.*, 2005). However, some authors have suggested the use of gas chromatography (GC) followed by mass spectrometry (MS) (HAWER *et al.*, 1994; KRAJEWSHA and POWERS, 1987; KRAJEWSHA and POWERS, 1988; MANIRAKIZA *et al.*, 1999; THOMAS *et al.*, 1998) and a recent report described coupled GC/MS with solid phase microextraction (SPME) (PEÑA-ALVAREZ *et al.*, 2009).

This paper presents an alternative and simple method to detect and quantify capsaicin, dihydrocapsaicin, and nordihydrocapsaicin with a Fast-GC system, which is useful for routine analysis. This method has not previously been proposed for the instrumental quantification of capsaicinoids. The AOAC Official Method 995.03 was used as the extraction method. GC/MS was adopted to confirm the identity of capsaicinoids in the start-up phase. The Fast-GC application demonstrated good peak resolution and the run time was comparable with that of various conventional and rapid HPLC techniques and lower than that corresponding to other GC conditions.

MATERIALS AND METHODS

Samples

Ten samples of dried ground red pepper from different origins were collected from import operators of the Italian market.

Reagents

Analytical-grade ethyl alcohol (99.8%) was purchased from Fluka Chemika (Sigma Aldrich-Milan, Italy). Capsaicin (>97%) and dihydrocapsaicin (90%) were purchased from Sigma Aldrich (Milan, Italy). Stock solutions of these capsaici-

noids were prepared by weighing and dissolving each compound in ethyl alcohol. These solutions were stored at 4°C and used for preparing diluted working standard solutions in ethyl alcohol.

Instruments and programmes

GC/MS. GC/MS analyses were carried out with a Shimadzu 2010 gas chromatograph coupled to a Shimadzu 2010 MSD quadrupole mass spectrometer (Shimadzu, Italy). Helium was used as the carrier gas at a flow rate of 1 mL min⁻¹. Suitable identification of the analytes was achieved using an Equity™-5, a poly (5% diphenyl - 95% dimethylsiloxane) stationary phase column, 30 m × 0.25 mm i.d., 0.25 µm film thickness (Supelco-Milan, Italy). The oven temperature programme was 60°C (held for 1 min) to 240°C at a rate of 3°C min⁻¹ (held for 5 min). The injector temperature was 230°C and the split injector mode (1:5) was used. The ion source and transfer line temperatures were 200° and 250°C, respectively. The MS operating conditions were: positive electron ionization mode (EI+) with automatic gain control, 70 eV of electron energy, and 250 mA of emission current. The mass spectrometer was operated in full scan mode from m/z 40 to 340. Capsaicin, dihydrocapsaicin, and nordihydrocapsaicin were identified by comparing the retention times and spectra of each capsaicinoid with those of standard compounds. The spectral data were obtained from the Di.Pro.Ve.-UNIMI homemade library.

Fast-GC. Fast-GC analyses were performed on a Shimadzu system composed of a GC 2010 gas chromatograph (Shimadzu Italia, Milan, Italy) equipped with a split/splitless injector, an autosampler AOC-20i, and a FID detector. Data acquisition was performed with GC Solution Software (Shimadzu Italia, Milan, Italy). Analyses were performed with a RTX®-5 (Crossbond® 5% diphenyl- 95% dimethyl polysiloxane) stationary phase column (Restek, Bellefonte, PA, USA). The column dimensions were 10 m × 0.10 mm i.d., 0.10 µm film thickness. The oven temperature programme was 180°C (held for 2 min) increased to 240°C at a rate of 8°C min⁻¹, then increased to 320°C at a rate of 14°C min⁻¹ (held for 5 min). The pressure programme (336 kPa) was a constant linear velocity. The velocity of the carrier gas (H₂) was 78.5 cm sec⁻¹. The injector temperature was 280°C and the split mode (1:100) was used. The injection volume was 1 µL. The detector parameters (FID) were: T = 340°C; H₂, 40 mL min⁻¹; Air, 400 mL min⁻¹; Makeup (N₂), 30 mL min⁻¹; Base period, 8 ms; Filter time constant, 50 ms; Sampling rate, 8 ms.

Extraction of capsaicinoids

Data were collected to determine the usefulness of the instrumental response of Fast-GC for quantitative evaluation of capsaicinoids to

calculate the SHVs for routine quality control. To prepare the extracts for Fast-GC evaluation, we used the AOAC Official Method 995.03 (1995). The filtered extract, produced by reflux of 25 g of ground or crushed red pepper with 200 mL of ethanol, was diluted 1:2 in ethyl alcohol prior to injection in Fast-GC.

Calibration and precision data

Once the Fast-GC procedure was optimised, calibration curves were created with standard solutions of the two standards of capsaicinoids commercially available, capsaicin and dihydrocapsaicin. Standard solutions were prepared in ethyl alcohol to cover concentrations up to 195 µg mL⁻¹ for capsaicin (i.e.: 3.0, 6.1, 9.8, 24.4, 48.8, 97.5, 146.2, and 195.0 µg mL⁻¹) and up to 105 µg mL⁻¹ for dihydrocapsaicin (i.e.: 2.6, 5.2, 13.1, 26.2, 52.5, 78.8, and 105.0 µg mL⁻¹). These concentration ranges were previously defined to be useful to calculate the content as mg kg⁻¹ of considered capsaicinoids in dried matrices studied by the concentration data of the alcoholic extract. The previous ranges cover the possible concentration of the extracts deriving from natural matrices: the factor multiplicative 16, deriving from the extraction method parameters, permits to express the capsaicinoid content in the matrix as µg g⁻¹ from the measured concentration µg mL⁻¹ in the extract. Regression equations and correlation coefficients were calculated using Microsoft Office Excel 2007 and are reported in Figure 1. The standard distribution data demonstrated a polynomial model for both capsaicin and dihydrocapsaicin in the range 3.0 - 195.0 mg mL⁻¹ for capsaicin and 2.6 - 105.0 mg mL⁻¹ for dihydrocapsaicin in the extracts analysed by Fast-GC. The data were fit with an R² = 0.996 for capsaicin and R² = 0.997 for dihydrocapsaicin and nordihydrocapsaicin. The calibration curve of dihydrocapsaicin was also used for evaluating nordihydrocapsaicin, as conventionally adopted by others (BARBERO *et al.*, 2006b; ZIINO *et al.*, 2009).

The limit of detection (LOD) and limit of quantitation (LOQ) values were respectively: 1.2 and 3.5 µg mL⁻¹ for capsaicin and 1.0 and 3.0 mg mL⁻¹ for dihydrocapsaicin and nordihydrocapsaicin. The repeatability and reproducibility were studied in respect to the concentration of each capsaicinoid. The average intraday relative standard deviations (RSD%), calculated on five samples shown in Table 1, were 3.9, 4.2, and 4.3 for capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, respectively. The average interday relative repeatability standard deviations (RSDr%), calculated on the ten samples shown in Table 1, were 5.4, 5.6, and 5.8 for the same three capsaicinoids. These data were determined with the NMKL procedure n.5 (NORDIC COMMITTEE ON FOOD ANALYSIS, 1997) for the estimation

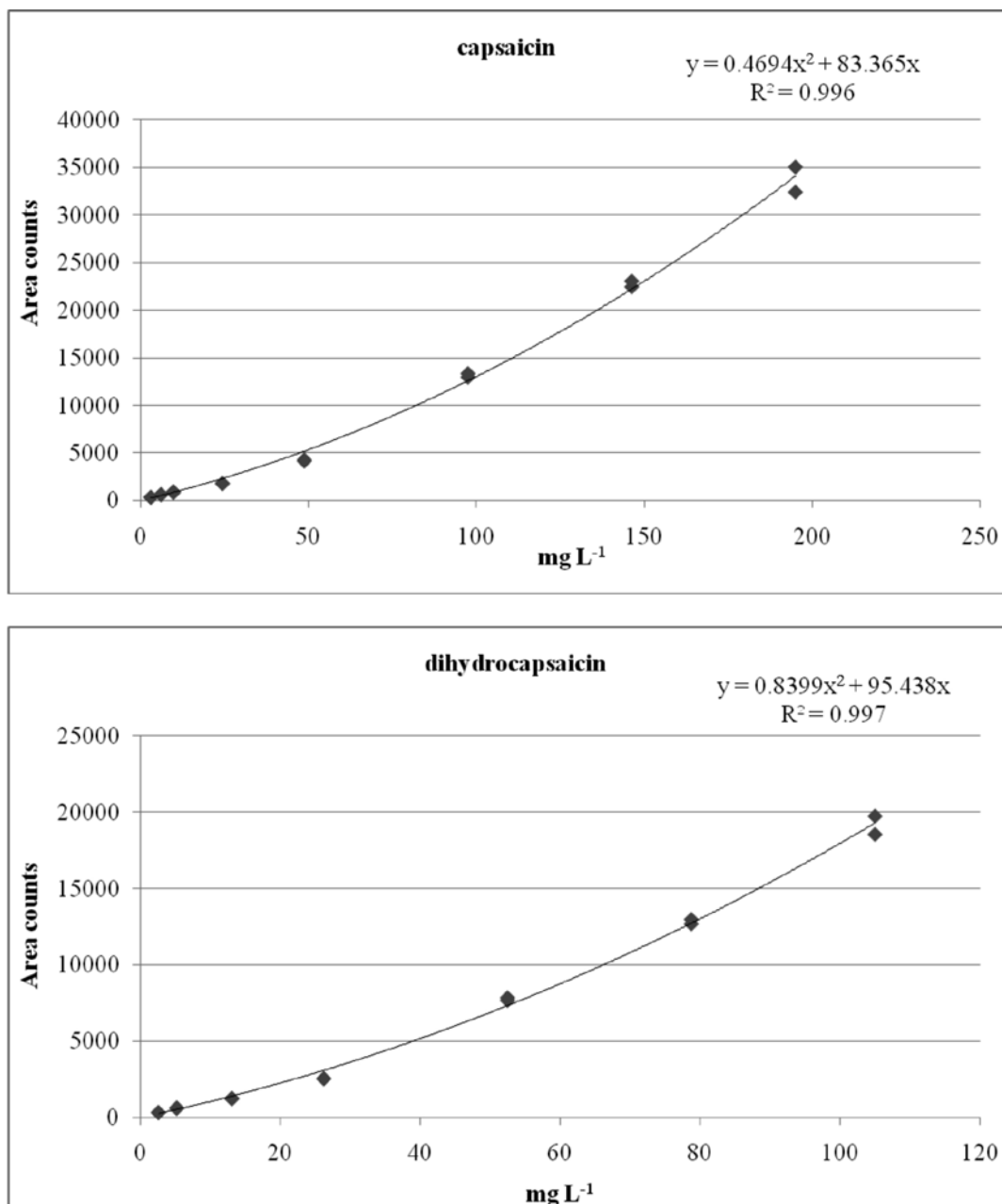


Fig. 1 - Calibration curves for capsaicinoids quantified with the Fast-GC method. The areas under the GC peaks were evaluated in counts (Y-axes) for each concentration of capsaicinoid (X-axes) in the extract. The data of both curves were fit (solid lines) with polynomial equations (upper right).

and expression of measurement uncertainty in chemical analysis.

Recovery data were estimated by spiking two samples (n. 4 and n. 5 in Table 1) having very different Scoville Heat Value, adding a calculated concentration of 500 mg kg⁻¹ for capsaicin and 200 mg kg⁻¹ for dihydrocapsaicin. The resulting recoveries were 98.6 and 97.7% respectively for capsaicin and dihydrocapsaicin for sample n.4 (mean of two determinations); the corresponding values for sample n.5 result 95,0 and 94,2%.

Statistical data performed by other GC methods

cited in bibliography don't show to be improved in respect of the ones reported in this paper.

RESULTS AND DISCUSSION

The Fast-GC method was used for quality control measurements in one company that produces a primary brand in the retail spices market. Ten different samples of *Capsicum* were tested with standard conditions and showed a wide range of SHVs (Table 1). Fast-GC equipment permitted good resolution of the

Table 1 - Scoville heat values (mean of two determinations) obtained for 10 commercial samples of *Capsicum* (different market samples) under standard operative conditions optimised for the Fast-GC method. The results cover a wide range of SHV values (mean of two determinations).

Sample	Capsaicin (mg kg ⁻¹)	Dihydrocapsaicin (mg kg ⁻¹)	Nordihydrocapsaicin (mg kg ⁻¹)	Scoville Heat Value SHV
1	366.7	244.9	270.2	12,360
2	4095.4	1901.6	373.5	100,028
3	1195.7	908.3	185.7	35,602
4	3742.3	1354.0	347.5	85,282
5	1028.4	610.1	138.9	27,673
6	3848.1	1823.1	352.6	94,585
7	847.0	603.4	157.3	25,619
8	1161.0	557.0	141.0	28,970
9	1353.3	945.7	209.2	38,960
10	3632.5	1679.1	363.0	88,892

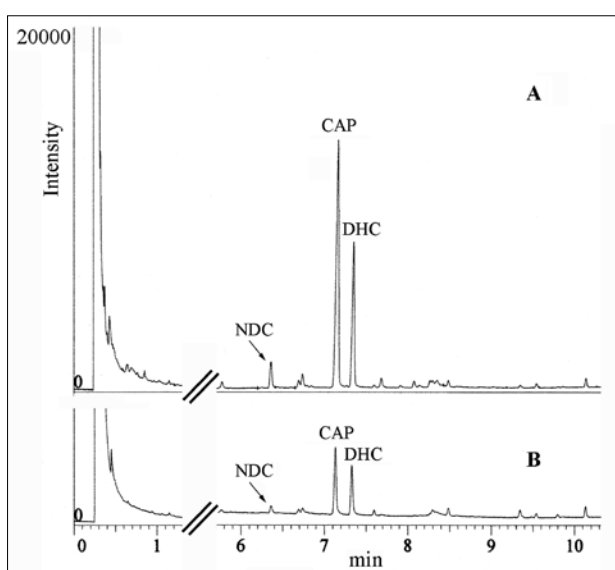


Fig. 2 - Fast-GC trace corresponding to the capsaicinoids, nordihydrocapsaicin (NDC), capsaicin (CAP), and dihydrocapsaicin (DHC), detected in pepper fruit samples. Samples 6 (A) and 7 (B) are characterised in Table 1. The SHV values are 94,585 and 25,619 for (A) and (B), respectively.

peaks of interest. Figure 2 shows two examples of resolution, A and B refer to samples 6 and 7, respectively, in Table 1 with SHVs of 94,585 and 25,619. Figure 2 shows that the identification and quantification of three capsaicinoids, using previously described standard operating conditions, were not difficult even for samples with very different SHVs. The primary advantage of Fast-GC quantitative determination was the rapidity of chromatographic elution. The time required for Fast-GC was comparable with the time required for other proposed HPLC methods, but shorter than that required by other currently proposed GC or GC/MS methods. These advantages were associated with RSD values below 5% and RSDr values below 6%.

CONCLUSION

We evaluated whether Fast-GC was useful for simultaneous analysis of three capsaicinoids commonly utilized for SHV determinations in pepper fruits. The results derived from this chromatographic system showed similar analysis times and good sensitivity and reproducibility compared to more frequently used HPLC-DAD methods. The Fast-GC technique, coupled to a proper extraction method, is particularly suitable for routine quality control. The total time of analysis depends on the preferred extraction technique but the time of Fast-GC analysis results very low (about 8 min) in comparison with the other corresponding GC chromatographic conditions.

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