ECO-FRIENDLY TECHNIQUE FOR TOMATOES VOLATILE FINGERPRINT DETERMINATION

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Abstract

In-tube extraction (ITEX) is a novel technique that can be coupled with gas-chromatography mass-spectrometry (GC-MS) and used for the extraction and separation of volatile compounds from different matrices. In this study a simple and efficient ITEX/GC-MS method was developed and optimized for the extraction and analysis of volatile compounds from tomatoes. The analytes were extracted from sample headspace by dynamic extraction and trapped into the selected sorbent material (Tenax TA), followed by their thermal desorption into the GC injector. Different extraction parameters were tested (incubation time and temperature, amount of sample, number of pumping strokes) and the optimal ones selected. The best compromised between the extraction performance and analysis time was achieved when 1g of sample was incubated at 70^{o}C for 20 min. followed by 30 extraction strokes. Between the main odor-active volatiles found in the analyzed cherry variety tomato samples were: hexanal, trans-2-hexenal, cis-3-hexenol, hexanol, 6-methyl-5-hepten-2-one, 2- and 3-methylbutanal, methyl salicylate, 1-penten-3-one.

Keywords: in-tube extraction, gas-chromatography – mass-spectrometry, method optimization, authenticity

INTRODUCTION

Headspace gas-chromatography mass spectrometry is an approach that offers an alternative to classical methods used for the determination of volatile compounds from different complex vegetable matrices and not only (Bicchi et al., 2008; Zhu and Chai, 2005; Zhang and Li, 2010). Headspace In-Tube Extraction Gas-Chromatography Mass Spectrometry (HS-ITEX/GC-MS) is a novel green technology that can be successfully used for this purpose. This technique requires no or minimal sample preparation, allowing a simple, efficient and rapid enrichment of volatile or semi-volatile compounds during the headspace analysis. The assembly consists in a sorbent bed placed between the needle and the body of the headspace syringe. After sample pre-conditioning (heating and shaking), using the plunger of the headspace syringe, the volatile compounds from the headspace are repeatedly pumped through the sorbent material leading to their concentration into the microtrap. The transfer of volatiles compounds

into the GC injector is then achieved by thermal desorption (Jochmann et al., 2008; Laaks et al., 2012; www.itex-headspace.com).

Since the commercialization of ITEX device in 2006 (by CTC Analytics AG, Zwingen, Switzerland) (Jochmann et al., 2008) there are several publications about the applications of this technique in different fields, such as: analysis of water contaminants (Laaks et al., 2010), analysis of low molecular weight organic compounds in blood and/or urine (Rasanen et al., 2010), or determination of highly volatile compounds from wines and beers (Zapata et al., 2012). All previous studies showed that ITEX technique can be successfully used for a rapid and sensitive determination of volatile compounds from a wide range of matrices. Also, it is an economical alternative to classical "purge and trap" techniques, with less instrumental effort and susceptibility to contamination. Although several hyphenated HS techniques were used for the determination of tomatoes volatile compounds (Lo Feudo et al., 2011; Serrano et al., 2009; Krumbein et al., 2004; Kovács et al., 2009; Farneti et al., 2012) to our best knowledge, ITEX technique wasn't one of them. We choose tomato as a vegetable matrix for this study, because tomato is one of the most consumed vegetable in the world, especially in the Mediterranean countries. Its importance for the human diet is due to its content in fibers, carbohydrates, proteins, lipids, minerals as well as several vitamins and anti-oxidants, namely lycopene, a carotenoid that plays an important role in the prevention of some diseases like prostate cancer (Georgé et al., 2011; Periago et al., 2009). The aim of this work was to develop a simple and efficient HS-ITEX/GC-MS method for the analysis of tomatoes volatile profile, which can be further use for cultivar characterization and authentication.

MATERIAL AND METHODS

Tomato samples

Table ripped tomatoes (*Lycopersicum esculentum*), cherry variety, originated from Spain, were purchased from a local supermarket. The samples were kept at -20^{o}C prior to experiments.

Extraction of volatile compounds

The extraction of volatile compounds was performed using ITEX technique. Briefly, a sample of mashed tomatoes was placed in a 20mL headspace vial and 1ml of saturated $CaCl_2$ solution per each gram of sample, was added. Using a CombiPAL AOC-5000 autosampler, the sealed vial was transferred into the agitation unit where it was incubated for the release of volatile compounds into the headspace of the vial. After incubation, an aliquot of gaseous phase was pumped repeatedly through a porous adsorption polymer fiber microtrap (ITEX-2TRAPTXTA, Tenax TA

80/100 mesh, ea) trapping the volatile compounds. The thermal desorption of volatiles was performed directly in the GC injector, and after, the hot trap (250^{o}C) was cleaned for 2 minutes with N_2. The following extraction parameters were tested: extraction time (10, 20, 30 and 40 minutes), extraction temperature (40, 60, 70 and 80^{o}C), weight of the sample (0.5, 1, 2 and 3g), number of aspirating and dispensing cycles (extraction strokes – 20, 30 and 40). All samples were analyzed in triplicate.

GC-MS analysis

The analyses were carried out on a GCMS QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) model gas chromatograph – mass spectrometer equipped with a CombiPAL AOC-5000 autosampler. The volatile aroma compounds were separated on a Zebron ZB-5ms capillary column (50m x 0.32mm i.d and 0.25µm film thickness). The carrier gas was helium, 1.39ml/min, injector temperature 250^{o}C. The used column oven temperature program was: 35^{o}C (hold for 10 min) to 50^{o}C at 3^{o}C/min to 150^{o}C at 6^{o}C/min, to 200^{o}C at 10^{o}C/min and hold for 5 minutes. The ion source temperature and interface temperature were set at 250^{o}C and the MS mode was EI. The mass range scanned was 35-350u. The identification of separated compounds was made based on the comparison of the obtained mass spectra with the ones from the mass spectra libraries, NIST27 and NIST147. All peaks found at least in two of the three total ion chromatograms (TIC) were taken into account when calculating the total area of peaks (100%) and the relative areas of the volatile compounds.

RESULTS AND DISCUSSION

The volatile compounds from tomato samples were extracted using ITEX technique and then separated by GC-MS. The most abundant volatiles detected in tomato fruits were those derived from lipids (linoleic and linolenic acids), such as: hexanal, *trans*-2-hexenal, *cis*-3-hexenol (Farneti et al., 2012). These compounds, together with hexanol, 6-methyl-5-hepten-2-one, methyl salicylate, 1-penten-3-one, 3-methylbutanol, 2 and 3-methylbutanal (also identified in tomato samples), positively contribute to the aroma of tomatoes, being important odor-active compounds for fresh tomatoes (Farneti et al., 2012; Buttery et al., 1987; Tandon et al., 2001; Yilmaz, 2001). Depending on the used extraction parameters, there were separated between 13 to 40 volatile compounds. Taking in consideration their importance for the aroma of tomatoes, five volatile compounds were selected (hexanal, *trans*-2-hexenal, *cis*-3-hexenol, hexanol, and 6-methyl-5-hepten-2-one) and their accumulation in the headspace phase of the vial was monitored, in order to optimize the extraction parameters for ITEX technique. For that purpose, after processing the chromatographic

fingerprint of the analyzed samples, the peak areas for each of the five selected compounds were obtained. The influence of the tested extraction parameters on these peak areas was then assessed.

Influence of the incubation time

The first optimized parameter was the time used to incubate the sealed vial containing 1g of tomato sample. The incubation periods were of 10, 20, 30 and 40 min., respectively. In all cases, the incubation temperature was 70^{o}C and after sample conditioning, 30 extraction strokes were performed. The trapped volatile compounds were then desorbed into GC injector and separated using the method described above. Figure 1 shows the relative accumulation of the selected compounds, depending on the incubation time. As it can be noticed, after 20 min. of incubation, for *trans*-2-hexenal, 1-hexanol and 6-methyl-5-hepten-2-one there is only a low increase of their amount in the headspace of the vial. Instead, for the major compound, hexanal, and also for *cis*-3-hexenol, a decrease in their accumulation in the headspace is observed, if the sample is incubated more than 20 min. From the point of view of the number of separated volatiles, when the tomato samples were incubated for 10 min., thirty compounds were separated. If the samples are incubated for 20 min. or more, the number of separated compounds increases to forty. The increase of incubation time from 20 to 30 or to 40 min. doesn't influence the number of separated compounds, no new compound being separated for an incubation time larger than 20 min. Also, the main volatiles were the same in all cases. Thus, for further investigation, the selected incubation time was of 20 min.

Influence of the sample amount

In order to see the importance of the sample weight for the extraction of the volatile compounds by ITEX technique, four different weights of tomato samples were analyzed: 0.5g (W0.5), 1g (W1), 2g (W2) and 3g (W3). All the other extraction parameters were kept constant (incubation temperature 60^{o}C, incubation time 20 min., extraction strokes 30), and the extracted volatile compounds were analyzed using the same GC-MS method as described previously. The same twenty-six volatile compounds were separated and identified in all samples, except W0.5 sample in which twenty-five volatiles were detected (2-pentyl-furan was under the detection limit for this sample). A consisted increase in peak areas, and thus in the compounds concentration in the headspace phase, is noticed when the sample weight increase from 0.5g to 1g, respectively to 2g in the case of hexanal.

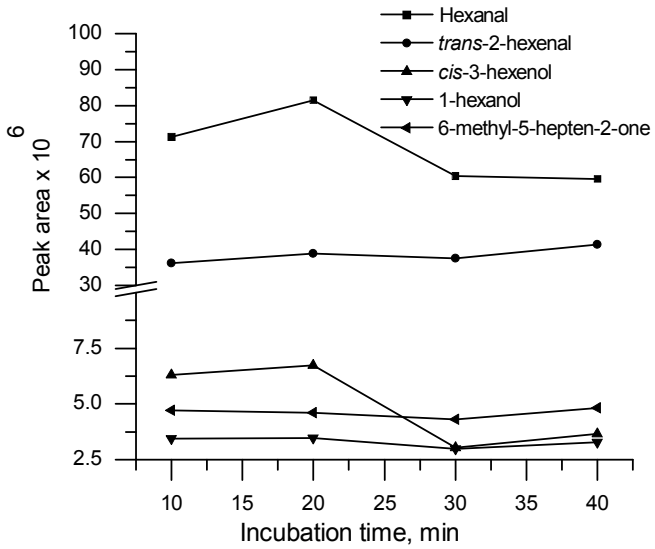

Fig.1. Influence of the incubation time on the obtained peak areas (from TIC chromatogram).

As it can be noticed from figure 2, when extracting the volatile compounds from sample amounts of 2g and 3g, no significant differences on peak areas of the selected compounds are observed. Therefore, it can be concluded that over a certain quantity of sample, some analytes were so concentrated in the headspace phase that the influence of the amount extracted onto the Tenax fiber was negligible. From the analysis of data obtained from the performed experiments, the optimal amount of sample would be of 2g. Although, to avoid the headspace phase saturation and thus the MS detector saturation, for further experiments, the selected sample amount was of 1g.

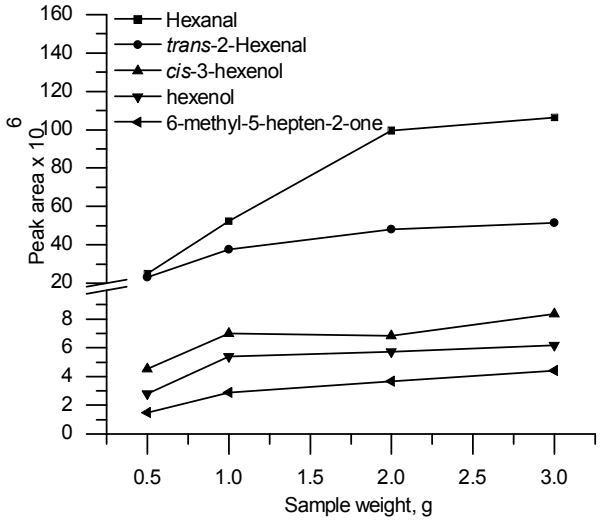

Fig.2. Influence of the amount of tomato sample on the obtained peak areas (from TIC chromatogram).

Influence of the extraction temperature

In order to optimize the extraction temperature, using a sample amount of 1g, several extraction were performed at each of the following temperatures: 40°C, 60°C, 70°C and 80°C. For the enrichment of Tenax trap in volatile compounds, 30 aspirating and dispensing cycles were carried out, for each experiment. At low extraction temperatures (40°C) only 13 compounds were separated according to the chromatographic data. Moreover, 1-hexanol, wasn't detected when the incubation of the sample was performed at this temperature. When increasing the extraction temperature to 60°C, an increase in the response of the analytes was observed (figure 3) but also a larger number of compounds was extracted and separated (18). Further increase of the extraction temperature to 70°C and 80°C, does not improve (except for hexanal and *trans*-2-hexenal) the accumulation of the volatiles in the headspace phase and their adsorption onto the sorbent material. When 80°C extraction temperature was tested, beside hexanal and *trans*-2-hexenal, for the other compounds a decrease of peak areas was observed. Also, this is a relative high extraction temperature and some oxidation reactions may occur, the resulting degradation compounds interfering with tomato aroma profile. This fact was also observed by other authors, when a similar technique (headspace-solid-phase microextraction, HS-SPME) was used for tomato aroma fingerprinting (Serrano et al., 2009). As a consequence, 70°C was selected as the optimal

extraction temperature and the following analyses were conducted using this extraction temperature.

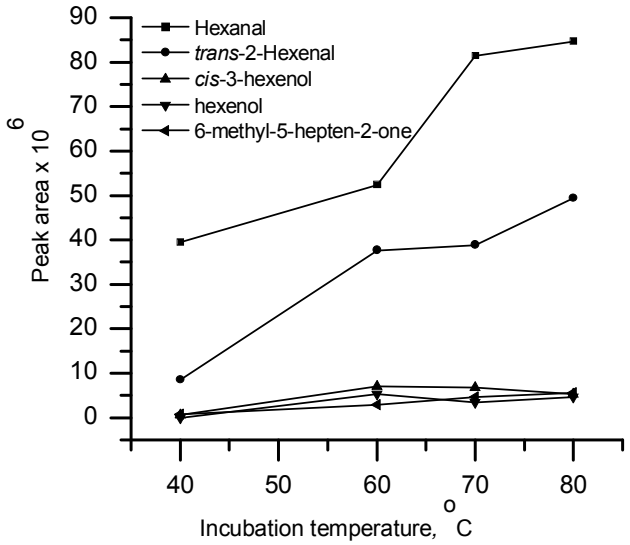

Fig.3. Influence of the incubation temperature on the obtained peak areas (from TIC chromatogram).

Influence of the aspirating and dispensing cycles (extraction strokes)

Finally, different numbers of aspirating and dispensing cycles were tested in order to further optimize the ITEX method. It is known that one of the main advantages of ITEX technique is that a higher sensitivity can be achieved by increasing the number of pumping strokes or by performing extraction from several vials containing the same sample (Hom and George, 2010; www.bgb-analytik.de). For that purpose, tomato samples (1g) were incubated at 70°C for 20 min., and from the headspace phase an aliquot was repeatedly pumped (20, 30 and 40 strokes) through the sorbent material. As expected, the higher the number of extraction strokes, the higher the amount of volatile compounds adsorbed and thus the higher the peak area (figure 4). An increase in the number of separated compounds from 33 to 40 was observed when the number of extraction cycles is increased from 20 to 30. Instead, when 40 extraction cycles are performed, no further increase in the number of separated volatiles was noticed, although the peaks of some minor compounds are sharper and the identification is more easily achieved. It has to be mention that when performing a higher number of extraction strokes, the total analysis time is also increased. Therefore, as a compromise (between the quality of extraction and the analysis time), the selected optimal number of aspirating and dispensing cycles was 30.

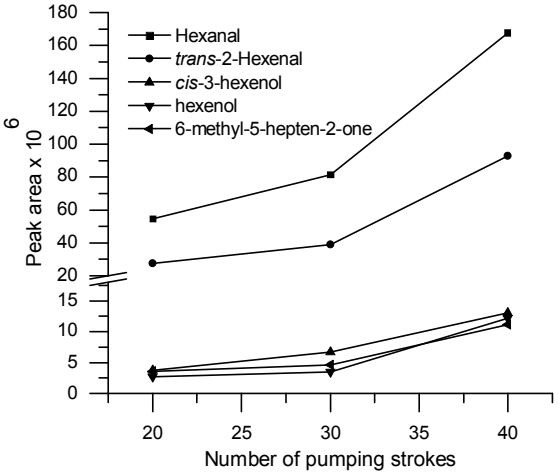

Fig.4. Influence of the number of the aspirating and dispensing cycles on the obtained peak areas (from TIC chromatogram).

CONCLUSION

An eco-friendly, solvent-free ITEX/GC-MS method for tomatoes volatile fingerprint determination was optimized. The optimal extraction was achieved when samples of 1g were incubated at 70^{o}C for 20 min., and then 30 aspirating and dispensing cycles were performed from the headspace phase for the enrichment of the microtrap with tomatoes volatiles. The main odor-active volatiles found in cherry variety tomato samples were: hexanal, trans-2-hexenal, cis-3-hexenol, hexanol, 6-methyl-5-hepten-2-one, 2- and 3-methylbutanal, methyl salicylate, 1-penten-3-one. The total time of analysis is around 1.5h per tomato samples, including the extraction of volatiles by ITEX technique and their gas-chromatographic separation. Also, one of the main advantages of the technique is that there is no need of sample preparation; the system is automized, and thus the cost/sample being considerably reduced. This method coupled with a chemometric approach will be further used for the discrimination and/or authentication of tomatoes varieties based on their volatile profiles.

Acknowledgements

The research was financially supported by a research grant offered by the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca and by Project PNCD II Ideas, contract no. 276/5.10.2011 and by the European Social Fund - The Operational Sectorial Program for Human Resource Development 2007 – 2013, project "Cellular and molecular biotechnologies for medical applications" FSE POSDRU/89/1.5/S/60746.

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