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Use of different sample temperatures in a single extraction procedure for the screening of the aroma profile of plant matrices by headspace solid-phase microextraction

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ABSTRACT

This study proposes a new approach to the optimization of the extraction of the volatile fraction of plant matrices using the headspace solid-phase microextraction (HS-SPME) technique. The optimization focused on the extraction time and temperature using a CAR/DVB/PDMS 50/30 μm SPME fiber and 100 mg of a mixture of plants as the sample in a 15-mL vial. The extraction time (10–60 min) and temperature (5–60 °C) were optimized by means of a central composite design. The chromatogram was divided into four groups of peaks based on the elution temperature to provide a better understanding of the influence of the extraction parameters on the extraction efficiency considering compounds with different volatilities/polarities. In view of the different optimum extraction time and temperature conditions obtained for each group, a new approach based on the use of two extraction temperatures in the same procedure is proposed. The optimum conditions were achieved by extracting for 30 min with a sample temperature of 60 °C followed by a further 15 min at 5 °C. The proposed method was compared with the optimized conventional method based on a single extraction temperature (45 min of extraction at 50 °C) by submitting five samples to both procedures. The proposed method led to better results in all cases, considering as the response both peak area and the number of identified peaks. The newly proposed optimization approach provided an excellent alternative procedure to extract analytes with quite different volatilities in the same procedure.

1. Introduction

Aroma is one of the most important attributes of fruit quality, and also the most significant quality parameter in comestible and processed products. From the point of view of the food and/or cosmetic industries, the determination of the aromatic profile of a plant matrix is of great concern. Knowledge of the aromatic profile of a plant enables improvements in the quality of the products and the development of new products for the market, as well as being of use in studies on the economical viability of either the essential oils obtained from plant extracts or the synthesis of major plant components.

Plant matrices generally contain compounds susceptible to decomposition through processes related to temperature, oxidation, photolysis, etc. Thus, sample preparation techniques which minimize both the sample manipulation and preparation times are highly desirable in order to achieve reliable results. These features can be achieved by using the headspace solid-phase microextraction (HS-SPME) technique [1,2]. HS-SPME coupled to gas chromatography has been successfully used for the determination of the aroma profile of plant matrices, including fig, melon, peach, apple, pepper, truffles [3–9] and others.

Notable among the commercially available extraction phases for SPME is the performance of CAR/DVB/PDMS and DVB/PDMS fibers for the extraction of the volatile fraction of plant matrices, which are the most frequently selected fibers [10–13]. This choice is probably due to the presence of PDMS (absorption mechanism) together with DVB and/or carboxen (adsorption mechanism). The presence of solid particles dispersed in PDMS is suitable for the retention of light molecules (volatiles).

The volatile fraction of a plant matrix is formed by a complex group of chemicals, including aldehydes, alcohols, ketones, esters, lactones, terpenes, etc. [14]. This means that to find a compromise extraction condition using HS-SPME for a complex mixture of compounds with relatively different volatilities/polarities can be considered an analytical challenge. Particularly in relation to extraction temperature, lower temperatures will favor the

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retention of the more volatile compounds. In contrast, higher temperatures will lead to an increase in the concentration of semi-volatiles in the sample headspace. Therefore, there will be a higher probability of extraction of these semi-volatile compounds by the coating, especially if, at this higher temperature, the equilibrium constants with the coating are favorable. Thus, an intermediate temperature is usually adopted as a compromise condition, with consequent lowering in the amount extracted for groups of both volatile and semi-volatile analytes.

In this regard, the objective of this study was to obtain a compromise condition for extraction of the volatile fraction of plant matrices in a single procedure, reaching the optimized condition for each group of analytes when considered separately. To achieve this, we carefully studied the variables extraction time and temperature by means of response surface methodologies using the CAR/DVB/PDMS fiber with separation/detection performed by GC–MS and GC–FID. A new optimization approach is proposed based on the use of two extraction temperatures in a single extraction procedure.

2. Experimental

2.1. HS-SPME procedure

A mixture containing 10 g of banana, 10 g of lemon zest, 10 g of basil and 10 g of parsley were mixed in a blender until complete homogenization. This mixture was transferred to a sealed glass flask and kept in the refrigerator at −15 °C. For the analysis, this mixture was removed from the refrigerator, and with the aid of a spatula 100 ± 5 mg was weighed directly into a 15-mL SPME (Supelco) vial together with a magnetic spin bar. The vial was tightly closed and immediately submitted to the extraction procedure. All experiments were carried out by exposing the CAR/DVB/PDMS 50/30 μm SPME fiber (Supelco) to the sample headspace, under constant magnetic stirring, without water addition and applying a pre-equilibration time of 5 min. In order to better understand how each variable affects the extraction efficiency of compounds with different volatilities/polarities, the chromatogram was divided into four groups of peaks based on the elution temperature: G_1 (40–88 °C), G_2 (89–135 °C), G_3 (136–183 °C) and G_4 (184–230 °C). For each group, the sum of the peak areas was used as the response, and for the compromise response surface the geometric mean of the four responses obtained for each group was calculated. It should be noted that in all cases a small sample mass (100 mg) was used in order to avoid fiber coating saturation (the fiber used contains solid particles dispersed in PDMS) and also to prevent saturation of the mass spectrometer detector, increasing its life-time. As the objective is to develop a very simple and reliable qualitative method for the screening of the volatile fraction of plant matrices, we decided to study only the factors extraction time and temperature, although it is well known that other variables such as water addition, salting-out effect and sample pH can interfere with the extraction process. Conversely, these variables can also produce artifacts, which are highly undesirable. Thus, we decided not to study other variables but only the extraction time and temperature.

The optimization strategy consisted of two steps:

(1) *Simultaneous optimization of extraction time and temperature*: Extraction time was evaluated in the range of 10–60 min, and extraction temperature in the range of 5–60 °C. In this step, the chromatogram was divided into four groups of peaks, and the sum of the peak areas for each group was used as the response to plot the response surfaces. Based on the results obtained for the optimum extraction temperature for each group, two temperatures (high and low) were chosen for use in the next optimization step. The high level of extraction time chosen for the experimental design (60 min) was based on a maximum time in which the sample throughput could be maintained acceptable. Extraction temperature high level (60 °C) was chosen in order to minimize or prevent the formation of artifacts.

(2) *Simultaneous evaluation of the total extraction time and the fraction of time at each extraction temperature chosen previously*: In this optimization step, the process always began with the high temperature (60 °C), which was held for a certain time according to the experimental design. After this time, the vial was removed from the bath at 60 °C and immediately transferred to the bath at 5 °C, in which it remained until the total extraction time required by the experimental design was completed.

The extracted analytes were thermally desorbed in the GC injector port at 240 °C for 8 min in splitless mode. No carry-over effect was observed.

2.2. Instruments

Optimization and application steps were carried out in a Shimadzu GC-14B gas chromatograph equipped with flame ionization detector and an Rtx-WAX (30 m × 0.25 mm × 0.25 μm) separation column obtained from Restek (Benner Circle, Bellefonte, PA, USA). The oven temperature was programmed as follows: 40 °C (4 min), 4 °C min^{-1} until 230 °C (1 min). Injector and detector temperatures were set at 240 and 260 °C, respectively. Ultrapure nitrogen was used as the carrier and make-up gas at 1.0 and 40 mL min^{-1}, respectively.

For identification purposes, a Shimadzu (Kyoto, Japan) GCMS QP-2010 plus gas chromatograph was used. It was equipped with an Rtx-5MS column (30 m × 0.25 mm × 0.25 μm) purchased from Restek. The column oven temperature was the same as that used for the GC–FID instrument. Helium at 1.0 mL min^{-1} was used as the carrier gas. The injector temperature was set at 260 °C. Interface and ion source temperatures were set at 250 °C.

3. Results and discussion

The extraction time in the range of 10–60 min and temperature in the range of 5–60 °C were evaluated using a central composite design. Fig. 1 clearly shows that the optimum conditions of extraction time and temperature were different depending on the analyte volatility. For instance, for the more volatile compounds in G_1 it can be observed that even temperatures lower than 5 °C lead to a significant increase in the amount extracted compared to higher temperatures. This result is to be expected, since the increase in the headspace concentration on increasing the sample temperature is not able to compensate the decrease in the equilibrium constants between the analytes and the coating. The behavior in relation to the extraction temperature for the compounds in groups G_2 and G_3, for which the optimal temperature ranged from 40 to 50 °C, is quite different from that of G_1. This is due to the lower volatilities of these compounds compared to those in G_1. The response surface for G_4 shows that there is a tendency toward an increase in the response as the extraction temperature is increased. However, temperatures higher than 60 °C were not evaluated in order to preserve the integrity of the analytes and sample.

In general, the optimal extraction conditions, especially temperature, are relatively different for each group, and any compromise condition adopted will deteriorate the extraction efficiency for one or more group of analytes. Fig. 2 shows the compromise response surface for all the analytes.

One can observe in Fig. 2 that the optimal extraction temperature considering all the compounds is around 50 °C and the optimal

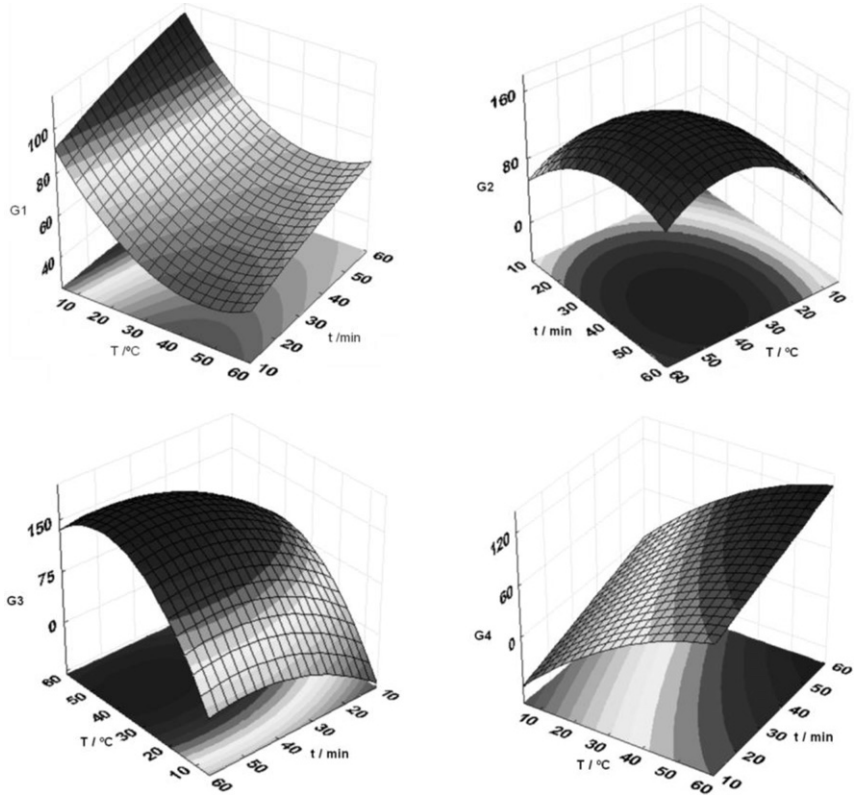

Fig. 1. Response surfaces obtained through the simultaneous evaluation of extraction time and temperature for each group.

extraction time seems to lie at some point over 60 min. However, taking into account a compromise between analytical frequency and sensitivity, 45 min of extraction was adopted.

Aiming at overcoming the problems related to the different extraction conditions for each group of analytes, a new approach based on the use of two extraction temperatures in a single procedure was studied. It is assumed that at higher temperatures the semi-volatiles will be preferentially extracted, and at lower sample temperatures more favorable conditions will be created for the extraction of the more volatile compounds. Based on the results in Fig. 1, high and low extraction temperatures were chosen: 60 and 5 °C. Fig. 3 shows the results obtained in the evaluation of total extraction time (the sum of extraction time at both sample temperatures) and the fraction of this time for which the sample temperature was carried out at 5 °C. The response used to plot Fig. 3 was the geometric mean obtained for the response of each group.

In order to allow a direct comparison between the newly proposed method and the conventional compromise extraction condition (45 min at 50 °C), the total extraction time chosen was 45 min (Fig. 3). At this total extraction time, the fraction of the

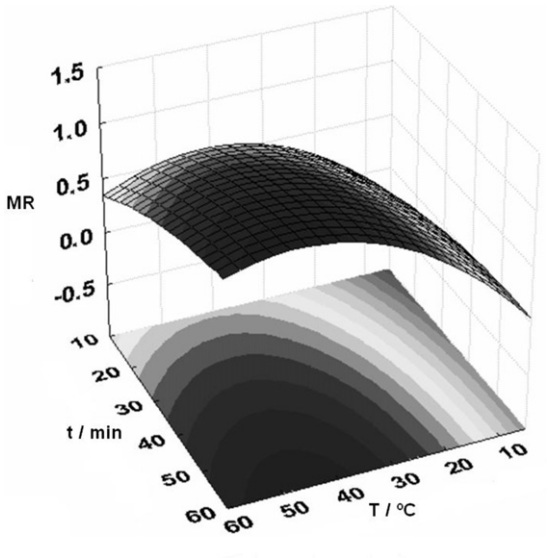

Fig. 2. Compromise response surface obtained in the simultaneous evaluation of extraction time and temperature. MR = mean response calculated as the geometric mean for the responses of the four groups.

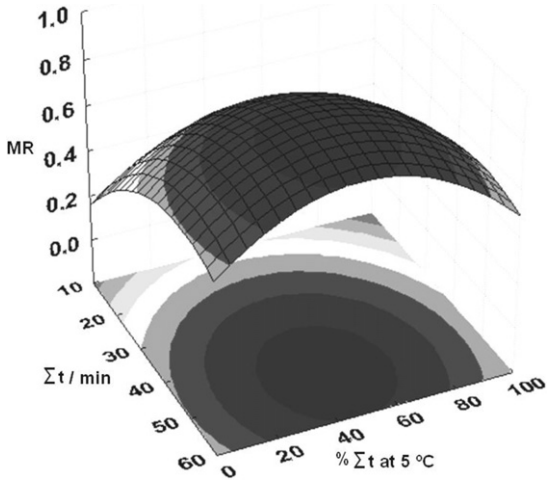

Fig. 3. Response surface obtained in the optimization of total extraction time (Σt = sum of extraction time at both extraction temperatures) and fraction of this time for which the extraction was carried out with sample temperature at 5 °C (% Σt at 5 °C). Initial and final temperatures were 60 and 5 °C, respectively. MR was defined in the caption of Fig. 2.

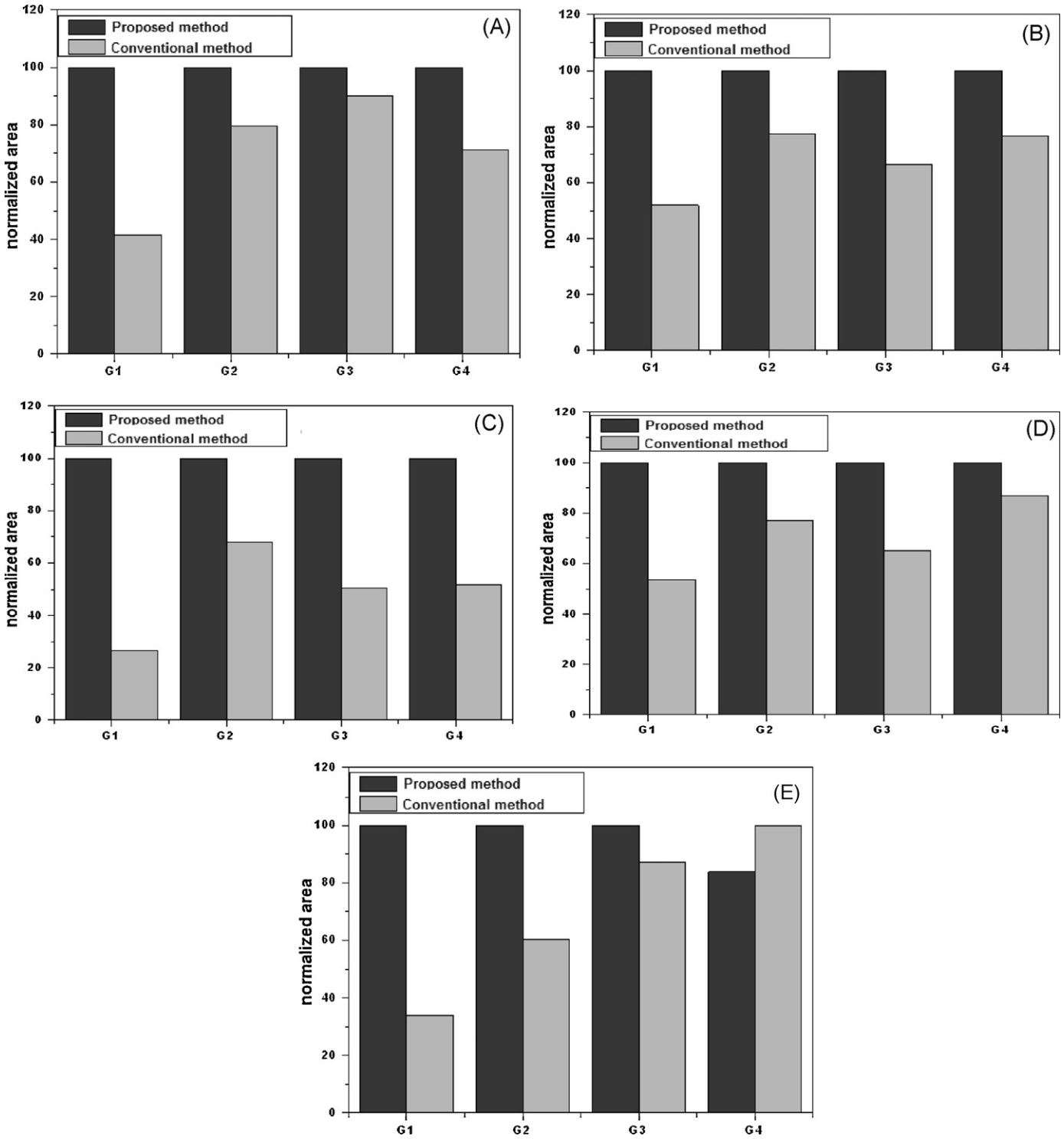

Fig. 4. Comparison between the proposed and conventional methods for the extraction of the volatile fraction of (A) banana, (B) passion fruit, (C) mango, (D) cherry and (E) toasted/ground coffee samples.

45 min for which the extraction should be performed at 5 °C is around 33%. Thus, the optimal extraction condition can be established as: 30 min of extraction at 60 °C, followed by a further 15 min of extraction at 5 °C.

The profile in Fig. 3 can be explained based on the results of Fig. 1. When the whole extraction procedure is performed at 5 °C or at 60 °C, the extraction efficiency is improved for one group of analytes in detriment to the other three. Thus, the use of two extraction temperatures seems to satisfy the optimal condition for different groups of analytes with distinct volatilities present in a certain sample.

In order to compare the two developed methods, five samples were submitted to both procedures: 45 min at 50 °C (conventional method) and 30 min at 60 °C followed by 15 min at 5 °C. Fig. 4 shows the results obtained for banana, passion fruit, mango, cherry and toasted/ground coffee samples.

It can be observed in Fig. 4 that the proposed method based on the use of two extraction temperatures is more efficient for

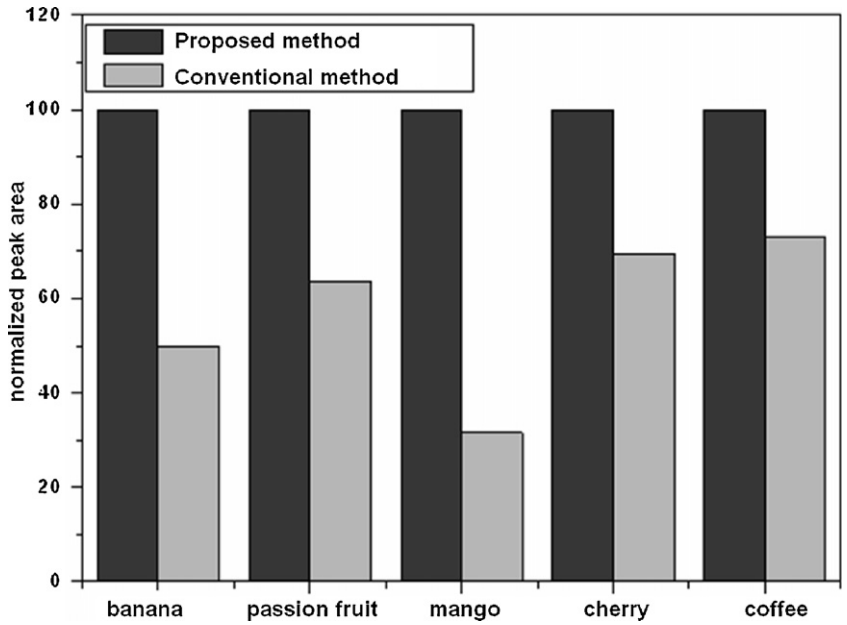

Fig. 5. Comparison obtained between the proposed and the conventional methods for the extraction of the volatile fraction of selected samples using as the response the total area obtained by GC–FID analysis.

the extraction of all of the compounds in all samples analyzed. Figs. 5 and 6 show the comparison using as the response the total area obtained by GC–FID (Fig. 5) or the summed area only of the peaks that could be identified in the GC–MS with similarity above 85% (Fig. 6).

As can be seen in Figs. 5 and 6, the procedure proposed in the study was shown to be more efficient regardless of the response considered.

Finally, Fig. 7 compares the number of peaks identified by the GC–MS instrument with a similarity between the two methods of above 85%. Again, the method based on the use of two extraction temperatures in a single procedure allows the analyst to identify a large number of compounds within a single sample.

This versatility in relation to the effective extraction of compounds with relatively different volatilities is due to the use of two different temperatures in the same procedure. During the period in which extraction is performed at the high temperature (60 °C), semi-volatile compounds are preferentially extracted, while the headspace is enriched by the most volatile. Once the sample temperature is decreased (5 °C), favorable conditions for the extraction of more volatile compounds are created. On the other hand, the semi-volatile compounds are more likely to undergo condensation and/or to partition back to the sample. During the process of cooling the sample from 60 to 5 °C, when condensation of a fraction of compounds present in the headspace occurs, there is a certain probability, though low, of these compounds condensing onto the

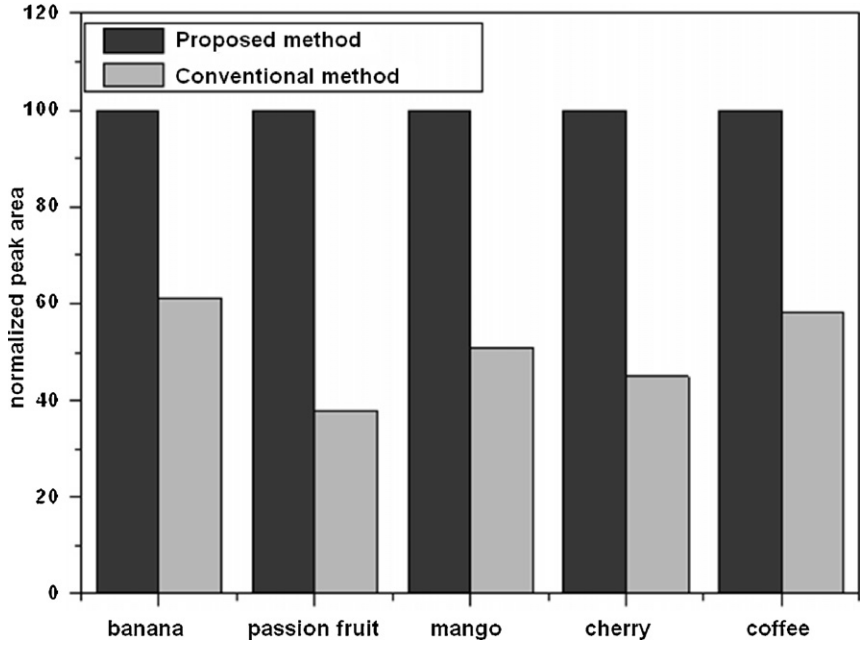

Fig. 6. Comparison obtained between the proposed and the conventional methods for the extraction of the volatile fraction of selected samples using as the response the summed area of the compounds identified in the GC–MS analysis with similarity above 85%.

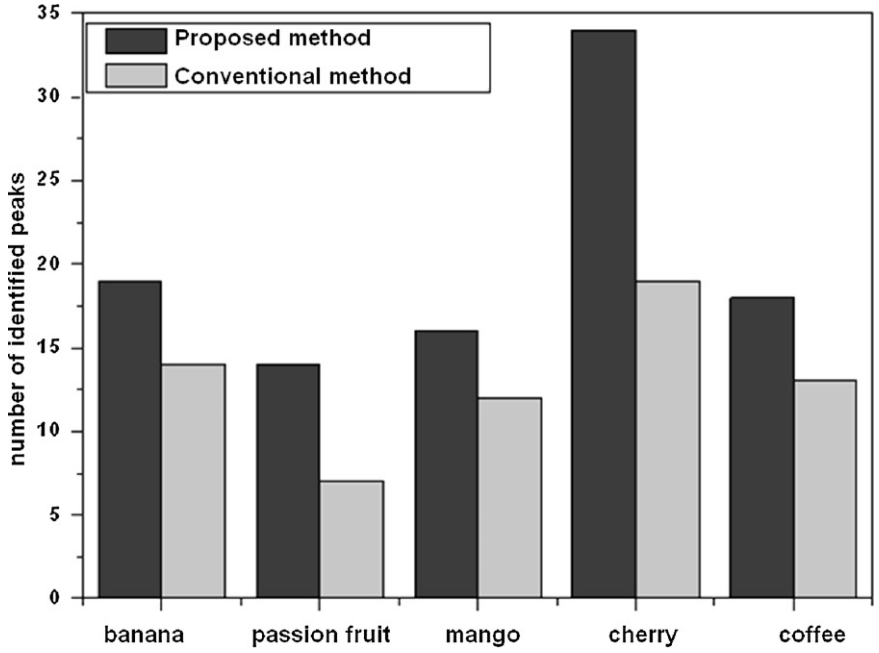

Fig. 7. Comparison between the proposed and the conventional methods for the extraction of the volatile fraction of the selected samples using as the response the number of peaks identified by GC–MS with similarity above 85%.

fiber coating surface, also contributing to the increase in the extraction efficiency compared to the conventional approach based on a single extraction temperature.

4. Conclusions

The new approach proposed in this study based on the use of two sample temperatures in the same HS-SPME procedure was demonstrated to be a very useful alternative to the conventional procedure. The proposed method extracts a larger amount of compounds with different volatilities and, importantly, allowed a greater number of compounds to be identified with a high degree of certainty using GC–MS analysis. In this study and for the samples analyzed, two extraction temperatures were found to be efficient. However, for the efficient extraction of a mixture of compounds with an even greater range of volatilities and sample complexity, the use of three or more temperatures may be required. Also, the development of a thermostatic bath equipped with temperature programming, in which it is possible to adjust the initial temperature, cooling rate and final temperature, could be used to facilitate the automation of this procedure so as to further increase its efficiency and applicability.

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