Development of a robust HS-SPME-GC-MS method for the analysis of solid food samples. Analysis of volatile compounds in fresh raw beef of differing lipid oxidation degrees

Mónica Bueno, Virginia C. Resconi, M. Mar Campo, Vicente Ferreira, Ana Escudero

PII: S0308-8146(18)32186-1
DOI: https://doi.org/10.1016/j.foodchem.2018.12.082
Reference: FOCH 24041

To appear in: *Food Chemistry*

Received Date: 6 July 2018
Revised Date: 10 December 2018
Accepted Date: 15 December 2018

Please cite this article as: Bueno, M., Resconi, V.C., Mar Campo, M., Ferreira, V., Escudero, A., Development of a robust HS-SPME-GC-MS method for the analysis of solid food samples. Analysis of volatile compounds in fresh raw beef of differing lipid oxidation degrees, *Food Chemistry* (2018), doi: https://doi.org/10.1016/j.foodchem.2018.12.082

Development of a robust HS-SPME-GC-MS method for the analysis of solid food samples. Analysis of volatile compounds in fresh raw beef of differing lipid oxidation degrees

Mónica Bueno [a], Virginia C. Resconi [b]; M. Mar Campo [b]; Vicente Ferreira [a]; Ana Escudero [a,*]

[a] Laboratory for Flavor Analysis and Enology (LAAE), Department of Analytical Chemistry, Faculty of Sciences, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (Universidad de Zaragoza-CITA), Associate unit to Instituto de las Ciencias de la Vid y del Vino –ICVV- (UR-CSIC-GR), 50009 Zaragoza, Spain.

[b] Department of Animal Production and Food Science, Faculty of Veterinary, Instituto Agroalimentario de Aragón (IA2) (Universidad de Zaragoza-CITA), 50013 Zaragoza, Spain.

* Corresponding author.

Tel.: +34 976 762503; fax: +34 976 761292; e-mail address: escudero@unizar.es

Abstract

This work presents a headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) method for the analysis of solid food samples in extended experiments. The final procedure was used to quantify 30 volatile compounds in fresh beef. The strategy adds robustness to the classic SPME methods for solid samples, by including a control solution that solves several challenges. The control solution contained one representative compound for each studied family of beef, and two internal standards. Response factors were calculated for each family, and were subsequently applied to every compound belonging to the same family. This strategy allowed control of the quantification procedure even when the fibre, column or control solution changed. Repeatability and reproducibility had relative standard deviation values below 17%, except for phenylacetaldehyde, (*E*)-2-nonenal and (*E*,*Z*)-2,4-decadienal. Although the method described here was applied to animal products, it has also been successfully used to distinguish between samples from different lipid oxidation stabilities.

Keywords

Beef aroma, volatile compounds, solid food samples, external control, direct analysis, HS-SPME-GC-MS.

1. Introduction

The volatile compounds responsible for beef aroma have been extensively studied in cooked samples, but less has been published about raw meat (Insausti, Beriain, Gorraiz, & Purroy, 2002; King, Hamilton, Matthews, Rule, & Field, 1993; Perez, Rojo, Gonzalez, & De Lorenzo, 2008). However, the volatile compounds released upon opening the package must be acceptable to the consumer, and how long the period of acceptability lasts will depend on numerous factors, such as the antioxidant status of the meat. Furthermore, some compounds which are already present or which develop in the raw meat will remain after cooking and affect the flavour perception (Insausti, Beriain, Gorraiz, & Purroy, 2002; Rota & Schieberle, 2005; Schindler, Krings, Berger, & Orlien, 2010).

Different techniques have been employed for extracting the volatile fraction of beef. In the present work, solid phase microextraction (SPME) sampling combined with gas chromatography and mass spectrometry (GC-MS) was selected to collect volatiles in the headspace (HS), because this technique provides information most closely matching what happens when a package of raw beef is opened. Although the limitations of SPME are known -- such as competition phenomena between volatiles in their adsorption of the SPME fibre, that may affect quantification (Met & Yesilcubuk, 2017; Oliver-Pozo, Aparicio-Ruiz, Romero, & Garcia-Gonzalez, 2015) -- it is a recognized technique for the analysis of volatile and semi-volatile compounds in beef (Acevedo, Creixell, Pavez-Barra, Sanchez, Albornoz, & Young, 2012; La Storia, Ferrocino, Torrieri, Di Monaco, Mauriello, Villani, et al., 2012; Machiels & Istasse, 2003; Saraiva, Oliveira, Silva, Martins, Ventanas, & Garcia, 2015) due to its many advantages: it is simple, cost-efficient, solvent-free, user-friendly; easily automated and implemented, and of high sensitivity. Moreover, because a low extraction temperature can be used, SPME gives a better estimation of the aroma profile as perceived by the human nose (Brunton, Cronin, Monahan, & Durcan, 2000). In the present case, fresh raw meat is being studied; therefore, a very rapid method is needed in order to minimise or prevent sample changes associated with enzyme activity, lipid oxidation, or microbial growth (Pawliszyn, 2009).

Comparisons among samples of solid complex matrices are usually found using just raw areas (Machiels & Istasse, 2003; Perez, Rojo, Gonzalez, & De Lorenzo, 2008; Saraiva, Oliveira, Silva, Martins, Ventanas, & Garcia, 2015) or area percentages (Bergamini, Wolf, Perotti, & Zalazar, 2010; Cordero, Bicchi, & Rubiolo, 2008; R. Costa, Fanali, Pennazza, Tedone, Dugo, Santonico, et al., 2015; Cullere, Ferreira, Venturini, Marco, & Blanco, 2013). In the first case, there is no control over changes related to fibre adsorption-desorption or to detector sensitivity. In the second case, a problem arises when all the compounds vary in the same way and, therefore, no differences between samples are observed (Bueno, Resconi, Campo, Cacho, Ferreira, & Escudero, 2013). The difficulty in quantification lies in the heterogeneity of solid samples, associated with unspecific variations in sample-gas and gas-fibre distribution coefficients. Furthermore, fibre has to be changed in experiments that last for a long period of time. Small variations in the fibre's coating have a direct impact on the number of molecules adsorbed on it. Therefore, SPME is extremely sensitive to matrix variations and variations between fibres, and so a control of the process must be implemented.

Problems might be solved by standard addition. However, in solid matrices the mass-transfer mechanism can be different for the standards added and the native analytes (Ouyang & Pawliszyn, 2008) due to differences in binding and adsorption sites (Mirnaghi, Mousavi, Rocha, & Pawliszyn, 2013). Addition of an internal standard (IS) could be another way of controlling the process. This will correct for instrumental response drifts only if the IS closely resembles the analytes in terms of affinity for the extraction phase and any competing phase in the matrix (Souza-Silva, Gionfriddo, & Pawliszyn, 2015). Therefore, a further consideration will be to find an appropriate IS with similar volatility and similar distribution coefficients, and no coeluting with any other compound (Machiels & Istasse, 2003) with the same mass-to-charge ratio. An isotopically labelled standard is the most suitable solution, but when a large number of molecules are required, their high cost causes one to consider other options. Moreover, to the best of our knowledge, direct addition of isotopic standards to meat samples has not yet been achieved.

In summary, the lack of control over the integrity of the samples, the generation and acquisition of the signals, and the quantification process in the analysis of raw solid food samples need to be studied in depth. To overcome these difficulties, the main goal of the work described in this paper was to develop and optimise a robust HS-SPME method over time, assuming fibre or even column changes. For that purpose, a control solution that contains internal standards, and that overcomes several challenges associated with analysis of meat, has been included. This method was applied to the study of 30 aroma volatile compounds in fresh raw beef.

2. Materials and methods

2.1. Animals and samples

This study used the left knuckles from 56 crossbred 12-month-old bulls, with a cold carcass weight of 231-340 kg and an intramuscular fat content in the *longissimus thoracis* muscle of 1.5-2.5 %. The animals were raised on the same farm and fed concentrates (based on maize, barley, and soya) and cereal straw *ad libitum*.. After slaughtering, the left knuckle from each animal was obtained, vacuum packaged and aged for 15 days in the dark at 3±1 ºC. Then, 0.6-cm thick steaks were obtained. Steaks from eight animals were used for method optimisation, and the rest of the 48 steaks were used to confirm the applicability of the method. The samples from day 0 of display were analysed on the day of sampling, whereas the rest were placed individually in trays, and sealed with a polyethylene and polyamide laminate film. Then trays were placed in simulated retail display (Koxka, V1VI1–5; Pamplona, Spain) under lights (cool white fluorescent illumination, 1200 lux, 16 h on, 8 off, Mazdafluor Aviva TF/36w; Philips, Eindhoven, Holland) at 4 ºC±1 ºC for 9 days.

Volatile compounds and lipid oxidation analyses were conducted in the *rectus femoris* muscle from the same steak. This muscle was minced and portions for each analysis were immediately taken. All analyses were conducted using fresh meat samples (not previously frozen).

2.2. Lipid oxidation and oxidative groups

Lipid oxidation was measured with the thiobarbituric acid reactive substances (TBARS) method (Pfalzgraf, Frigg, & Steinhart, 1995). TBARS values were calculated from a daily standard curve of 1,1,3,3-tetramethoxypropane (TMP, Sigma Aldrich), and expressed as mg malondialdehyde (MDA)/kg sample. Based on the results obtained at day 9 of display, samples were grouped according to the extent of lipid oxidation, into low (less than 1 mg MDA/kg), medium (TBARS values between 1-2 mg MDA/kg) and high oxidative samples (TBARS value exceeded 2 (Campo, Nute, Hughes, Enser, Wood, & Richardson, 2006)). Thus, each animal was allocated a single level of oxidation.

2.3. Reagents, standards and materials

Ethanol LiChrosolv quality, dichloromethane SupraSolv quality and hexane Unisolv quality were supplied by Merck (Darmstadt, Germany). Isooctane 99%, 1,2-propanediol 99.5% and glycerine 99.5% were purchased from Panreac (Barcelona, Spain). Dipropylene glycol 99% was supplied by Alfa Aesar (Karlsruhe, Germany).

The standards used for the identification, control compounds, and internal standards (IS) for the control solution were supplied by Aldrich (Madrid, Spain), Fluka (Madrid, Spain), and Lancaster Synthesis (Eastgate, UK). Chemical standards (analytical reagent grade): 2-pentylfuran ≥98%, 1-hexanol 98%, 1-octen-3-ol 98%, 2-butanone >99%, 2-heptanone ≥98%, 2-octanone ≥98%, 3-octanone ≥98%, 2-nonanone ≥99%, 1-octen-3-one >99%, 3-octen-2-one ≥98%, 3-nonen-2-one 95%, hexanal 98%, heptanal ≥95%, nonanal 95%, phenylacetaldehyde >95%, (E)-2-heptenal ≥95%, (E)-2-octenal 94%, (E)-2-nonenal 97%, (E)-2-undecenal ≥95%, (E, E)-2, 4-nonadienal ≥85%, (E, E)-2, 4-decadienal ≥89%, butanoic acid 99.5%, pentanoic acid 98%, hexanoic acid 99.5%, heptanoic acid 99%, octanoic acid 98%, nonanoic acid ≥96%, decanoic acid ≥98%. The compounds (Z)-2-octenal and (E,Z)-2,4-decadienal were found in commercial (E)-

2-octenal and (E,E)-2,4-decadienal, respectively. Control solution IS (analytical reagent grade): methyl 2-methylbutyrate ≥98%, 2,6-dichloroanisole (DCA) >97%.

Divynilbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS 1cm) 50/30 μm film thickness, polydimethylsiloxane/divynilbenzene (PDMS/DVB) 65 μm film thickness, and carboxen/polydimethylsiloxane (CAR/PDMS) 75 μm film thickness SPME fibres were purchased from Supelco-Spain (Madrid, Spain).

2.4. Method development

Samples with different levels of oxidation or a different number of display days were chosen based on the requirements of the experiments needed to develop the method.

2.4.1. Fibre selection

The three aforementioned fibres were tested. The best fibre was chosen by comparing the means of the peak areas of the different compounds from day 0 of display (less oxidised) and day 9 of display (more oxidised) meat samples from the same animal using a *t* test. The chromatograms were obtained in duplicate. The extraction time was 60 min for these analyses.

2.4.2. Extraction time

HS-SPME sampling was performed in duplicate using samples with lipid oxidation values higher than 2 mg MDA/kg. Samples came from the same animal and were analysed at various extraction times (20, 40 and 60 min) with the chosen fibre to determine the best conditions for this work. Means of the peak areas were compared in two ways, globally or peak by peak using a *t* test.

2.4.3. Identification of important volatile compounds

The identity of 30 odorants was determined through the mass spectra and the linear retention indices (Table 1), and confirmed by injection of the pure reference standards when available. An alkane solution (C8-C28), 5 mg/L in dichloromethane, was employed to calculate the linear retention index (LRI) of each analyte.

2.4.4. Control solution in dipropylene glycol

2.4.4.1. Internal standards selection

First, candidates for internal standards were tested to ensure no coelution occurred with control compounds added in the control solution, and to ensure a gaussian peak shape. Candidates were tested by repeated analysis for several weeks. The internal standards data that were finally chosen are given in Tables 1 and 2.

2.4.4.2. Control compound selection

Control compounds were selected representing all the families of target compounds found in beef., Those which were better distributed along the entire chromatogram were chosen: butanoic acid (10 mg/L, acids), nonanal (20 mg/L, saturated aldehydes), (*E*)-2-nonenal (3 mg/L, alkenals), 3-octanone (2 mg/L, saturated ketones), 1-octen-3-one (2 mg/L, unsaturated ketones), 1-hexanol (80 mg/L, alcohols) and 2-pentylfuran (10 mg/L).

2.4.4.3. Control solution stability

Methyl 2- methylbutyrate (30mg/L) was used to calculate the stability of 1-hexanol, 3-octanone, 1-octen-3-one and 2-pentylfuran while 2, 6-dichloroanisole (15mg/L) was used for butanoic acid, nonanal and (*E*)-2-nonenal.

The internal standard was used to calculate relative areas of control compounds (equation 1). Mass correction is necessary because different control solutions could be slightly different in their amounts of compounds or internal standards.

Equation 1:

$$Mass\ corrected\ relative\ area = \frac{Area\ (compound\ 1) \Big/ mass\ (compound\ 1)}{Area\ (internal\ standard) \Big/ mass\ (internal\ standard)}$$

To verify the stability of the control solution, different vials containing 2 mL of the aforementioned solution were analysed using the same SPME fibre. Eight of them were prepared at the same time and put in the sampler tray at room temperature (25 ˚C). Another eight vials were prepared once a day and put in the sampler tray immediately before their analysis. In both cases, the control solution was tempered at room temperature for 20 min before the preparation of the vials. A *t* test compared whether the slope of the regression line of each control compound differed significantly from 0.

2.4.4.4. Control solution robustness

The GC-MS method used must guarantee the quality of the results throughout a long-term experiment in which it is necessary to make quantitative comparisons between samples. We must ensure that there are no significant changes in the system over time that would lead to an uncontrolled sampling or quantification. It is necessary to observe the stability of the instrument, maintaining the values at the desired levels over long periods of time. For this purpose, Shewhart control charts of the mass corrected relative area to the internal standard (equation 1) were developed.

The same control solution was analysed 3 times a day over 5 days with the same fibre, in order to obtain the average and the standard deviation (Table 1. Supplementary material) for the control charts. These charts were used to control the response of the system (GC-MS instrument + fibre) after using different control solutions, fibres or columns.

2.5. HS-SPME-GC-MS

For each steak, 4 g (± 0.001) of minced meat was transferred to a standard headspace vial. Volatile compounds in the headspace were preconcentrated on a PDMS/DVD fibre keeping the sample unstirred at 37 °C for 40 min, and were further analysed on a GC–MS equipped with a DB-WAXETR capillary column (60 m × 0.25 mm I.D., a film thickness of 0.25 μm from J&W Scientific, Folsom, CA, USA), and preceded by a 3 m × 0.25 mm uncoated (deactivate, intermediate polarity) precolumn from Supelco-Spain. The complete procedure can be found in the Supplementary Material S1 (HS-SPME proposed method details) and S2 (Gas chromatography-mass spectrometry details).

2.6. Control of the samples

The quantification of the compounds in the samples using selective mass (Table 1) was carried out applying a response factor obtained with a dipropylene glycol control solution analysed by GC-MS every 16 meat samples. This control solution contained a compound of each family (see section 2.4.4.2), and the response factor was calculated for each one of these compounds. This response factor was subsequently applied to all compounds of the same family.

2.6.1. Sample stability in the sampler tray

To verify the stability of the samples in the sampler tray, eight low oxidative group samples (<1 mg MDA/kg) from the same animal were analysed covering a range of 24 h. Another eight low oxidative group samples from another animal were analysed covering the same time range, but in this case, the vials were taken out of the fridge (4 °C) immediately before their analysis. Results were compared (in percentages) with those of non-nitrogen purged low oxidative group samples from a third animal.

2.6.2. Repeatability and reproducibility

With the goal of avoiding the animal effect, repeatability and reproducibility were carried out analysing samples from the same minced knuckles, one steak for each experiment (medium oxidative group, values between 1-2 mg MDA/kg).

Five samples were analysed with the same fibre and compared with the same control solution for repeatability.Four samples were analysed with four different fibres and related to four different control solutions to obtain reproducibility.

2.7. Data analysis

Correlation studies between TBARS results and μg/g of volatile compounds were directly carried out with Excel 2013 (Microsoft, Washington, USA). Samples from day 9 of display of the three different oxidative groups, low (n=16), medium, (n=15) and high (n=17) and the 16 correlated compounds (p<0.05) were used for the principal component analysis (PCA), carried out using Unscrambler vs. 9.5 from Camo (Norway).

3. Results and discussion

3.1. Chromatographic and headspace sampling conditions

Several fibre coatings are commercially available for extraction of volatile compounds. In this case PDMS/DVB, CAR/PDMS and DVB/CAR/PDMS were selected because they are able to extract a broader range of volatile compounds. More than 140 volatile compounds were separated and detected, with the highest number of detected compounds coming from the use of PDMS/DVB (175 compounds). However, a more interesting approach was to compare less oxidised and more oxidised meat. Here, the number of significantly different compounds (p<0.05) when comparing less oxidised and more oxidised meat ranged from 39 to 42 for the three different fibres. The results revealed that the PDMS/DVB and DVB/CAR/PDMS

were the most suitable for extraction of volatile compounds from beef, as has been observed elsewhere (Acevedo, Creixell, Pavez-Barra, Sanchez, Albornoz, & Young, 2012). However, PDMS/DVB was chosen for two reasons. It was the one which extracted the greater number of target aroma compounds selected, having regard to the scientific literature of raw beef (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015; Insausti, Beriain, Gorraiz, & Purroy, 2002; Panseri, Chiesa, Biondi, Rusconi, Giacobbo, Padovani, et al., 2015; Perez, Rojo, Gonzalez, & De Lorenzo, 2008; Saraiva, Oliveira, Silva, Martins, Ventanas, & Garcia, 2015; Schindler, Krings, Berger, & Orlien, 2010), and the intensity of oxidation-related carbonyl compounds was higher in the chromatograms obtained with this fibre, a result also observed for other matrices (Bueno, Zapata, & Ferreira, 2014).

The extraction time was chosen after preliminary experiments. Taking into account the area of all the target compounds, there were no significant differences between extractions of 60 min and 40 min. On the other hand, a significant decrease (p<0.05) was observed when extracting for only 20 min. Regarding these results peak to peak, a significant decrease (p<0.05) was found for 9 compounds at a 20 min extraction time, whereas for the 40 min extraction time only a decrease for (*E*)-2-undecenal was significant (p<0.05). As the declared objective of the present work is to develop a rapid HS-SPME method for quantifying some relevant volatile compounds in fresh raw beef in order to minimise or prevent changes in samples, an extraction time of 40 min was chosen. Ion peak chromatograms of the target analytes in fresh raw beef are shown in Figure 1.

3.2. Control solution development

Regarding external control solution development: first, it was necessary to find a matrix whose aroma compound volatility was as similar as possible to that of meat. Volatile organic solvents, such as ethanol, isooctane, dichloromethane, and hexane could not be used because they compete for the fibre and also produced large peaks in the chromatograms that co-eluted some compounds of interest. Next, in order

to avoid competition phenomena in the fibre, non-volatile organic solvents often used in cosmetics, such as 1,2-propanediol, glycerine and dipropylene glycol (P. Costa, Velasco, Loureiro, & Rodrigues, 2016), were tested. The release of volatile compounds over time was more stable in dipropylene glycol (DPG) than in the other solvents. For that reason, DPG was the chosen solvent for the control solution.

Another important feature was the selection of control solution internal standards. Methyl 2-methylbutyrate and 2,6-dichloroanisole fulfilled the condition of having a constant headspace signal over time (relative standard deviation, RSD < 4%, >3 months), so they were used for monitoring control compounds stability.

3.3. Control solution stability

With respect to control solution stability, results showed that vials could be left in the sampler tray at room temperature (25 ˚C) more than 19 hours, more than enough to assess meat samples in a day. Furthermore, the slope of the representation of the mass corrected relative areas of all the compounds did not significantly differ from 0 at least in the first 10 days. Hence, a new control compound solution was prepared every 10 days.

3.4. Interpreting control charts

A visual inspection of the control charts for each compound revealed the stability of the signal obtained in the instrument, and also provided information on possible trends and systematic behaviour. If the points were randomly distributed within the warning limits, the signal was considered stable. Figure 2 shows an example of a control chart created using six control solutions, five fibres and two columns.

On the other hand, the presence of a point outside the control limits or three consecutive points outside the warning limits indicated a lack of stability in the signal. At that moment the analysis of samples was stopped and a thorough check of the whole instrument (cleaning the injector, changing septum, changing

precolumn, cleaning the ion trap…) was done before the instrument could be used again; in other words, until the control solution analysis fell inside the warning limits. In the same way, when a fibre had to be changed, the same operation was performed. During the development of this method, several fibres were used, ranging from 8 to 236 extractions. The average of the extractions carried out with the same fibre was 150.

Regarding the robustness of the system using the control solution, it can be said that, despite changing the solution itself, the fibre or even the column, relative standard deviation percentages were maintained below 12 % for the compounds present in the solution (Table 1. Supplementary material).

3.5. Stability of the samples

As meat from three animals was needed for this experiment, the relative percentage (area percentage in the chromatogram at the initial moment is 100%) was calculated in order to compare the results. Figure 3 shows the effect of time in the relative percentage of several compounds in the analysis of vials kept in different conditions. The first set was not purged with nitrogen, the second (nitrogen-purged) was kept directly in the sampler tray and the third (nitrogen-purged) was taken out of the fridge immediately before analysis.

As shown in figure 3a for 3-octanone, the percentage increased with time, as expected, in the three sets of experiments. It may be concluded that oxygen is the agent responsible for such increases. Similar effects were observed for most aldehydes, ketones, and acids, and examples are given in figures 3b and 3c for (*E*)-2-nonenal and butanoic acid respectively. It was observed that purged-nitrogen samples could be left in the tray for at least eight hours and kept for 24 hours in the fridge. Without the purge step, oxidation processes occurred faster, resulting in an undesirable increment in some volatiles such as aldehydes, ketones, and acids, in line with results from pig fat (Fischer, Haas, Leppert, Lammers, Horner, Wuest, et al., 2014). Besides this, different patterns can be observed in figure 3, linear for 3-octanone and

(*E*)-2-nonenal while the oxidation of butanoic acid shows an exponential evolution. Oxidation of the latter goes further, and linear pattern of 3-octanone and (*E*)-2-nonenal could be simply the initial part of the same trend.

3.6. Repeatability and reproducibility

Repeatability was estimated using the same fibre and the same control solutions, and reproducibility using different fibres and different control solutions. Hence, the data were obtained assuming the best and worst possible scenarios; in other words, the scenarios with less and more variability. As expected, reproducibility values are greater than repeatability due to the modification of two factors, fibre and the control solution.

As can be seen in table 2, method precision was mostly satisfactory except for acids, where relative standard deviations reached values of 30%. Leaving aside these compounds, relative standard deviation values were below 17%, except for phenylacetaldehyde, (*E*)-2-nonenal and (*E*, *Z*)-2,4-decadienal.

In addition, it is to be expected that the acids would have the worst values of RSD since these compounds elute at the end of the chromatogram and have more time to diffuse, which causes a widening of the peaks that affects their integration.

Even though deviations of 30% could seem rather high when compared to the variability obtained between animals subjected to the same treatment (Virginia C. Resconi, Bueno, Escudero, Magalhaes, Ferreira, & Mar Campo, 2018), the variability of the method is appropriate (see Figure 1 in Supplementary material).

3.7. Application to samples with different oxidation levels

The method was applied to the study of 48 different beef steaks from different animals. The reproducibility of the method is enough to segregate samples from different degrees of oxidation (Figure

4). As shown in the PCA plot, the sixteen compounds have positive loadings on the PC1, which means that this component arranges beef samples by taking account of their general ability to produce these compounds upon oxidation, above all, alkenals and acids. The samples are ordered from low (circles) to high (triangles) oxidation levels. PC2 separates beef samples by taking account of their individual abilities to form saturated ketones (positive values of PC2) and unsaturated ketones and 1-hexanol (negative values of PC2). In fact, all the compounds appeared in all the samples. This makes sense because the knuckles had been aged for 15 days and then the samples were exposed to 9 days on display. Therefore, all the samples were oxidised, but to different degrees. The funnel shape of the plot means that the relative proportion of the measured compounds is not the same, and differs depending on the degree of lipid oxidation. The greater the degree of lipid oxidation, the further the samples can be placed on the extremes of PC2, producing more saturated and unsaturated ketones. Given these results, more complex studies on fresh raw beef meat can be carried out using this methodology (Virginia C. Resconi, Bueno, Escudero, Magalhaes, Ferreira, & Mar Campo, 2018).

4. Conclusions

This study describes the successful development of a robust HS-SPME-GC-MS method for the analysis of solid food samples in extended experiments . For this purpose,we have studied a dipropylene glycol control solution containing one representative compound of each studied family and two internal standards for monitoring control solution stability. This control solution is able to control the quantification procedure even if the fibre, the column or the control solution changed, with RSD (%) below 12% in the control solution. The final procedure can provide satisfactory measurements of 30 volatile compounds on fresh raw beef meat samples. Repeatability and reproducibility have RSD values below 17% except for phenylacetaldehyde, (*E*)-2-nonenal, (*E*,*Z*)-2,4-decadienal and acids. Nevertheless, the variability obtained between animals subjected to the same treatment is much higher than the one provided by this

strategy. Therefore, this methodology can be used to differentiate between samples from animals with different lipid oxidation stabilities.

Acknowledgements

V.C. Resconi was supported by a contract from the Juan de la Cierva Incorporación Program (Ministerio de Economía y Competitividad), Spain. Funding from Diputación General de Aragón (T53 and A04; PI046/08) and Fondo Social Europeo is acknowledged.

References

Acevedo, C. A., Creixell, W., Pavez-Barra, C., Sanchez, E., Albornoz, F., & Young, M. E. (2012). Modeling Volatile Organic Compounds Released by Bovine Fresh Meat Using an Integration of Solid Phase Microextraction and Databases. *Food and Bioprocess Technology, 5*(6), 2557-2567.

Almela, E., Jose Jordan, M., Martinez, C., Antonio Sotomayor, J., Bedia, M., & Banon, S. (2010). Ewe's Diet (Pasture vs Grain-Based Feed) Affects Volatile Profile of Cooked Meat from Light Lamb. *Journal of Agricultural and Food Chemistry, 58*(17), 9641-9646.

Bergamini, C. V., Wolf, I. V., Perotti, M. C., & Zalazar, C. A. (2010). Characterisation of biochemical changes during ripening in Argentinean sheep cheeses. *Small Ruminant Research, 94*(1-3), 79-89.

Brunton, N. P., Cronin, D. A., Monahan, F. J., & Durcan, R. (2000). A comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey. *Food chemistry, 68*(3), 339-345.

Bueno, M., Resconi, V. C., Campo, M. M., Cacho, J., Ferreira, V., & Escudero, A. (2013). Effect of freezing method and frozen storage duration on odor-active compounds and sensory perception of lamb. *Food Research International, 54*(1), 772-780.

Bueno, M., Zapata, J., & Ferreira, V. (2014). Simultaneous determination of free and bonded forms of odor-active carbonyls in wine using a headspace solid phase microextraction strategy. *Journal of Chromatography A, 1369*, 33-42.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*(2), 303-311.

Casaburi, A., Piombino, P., Nychas, G. J., Villani, F., & Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology, 45*, 83-102.

Cordero, C., Bicchi, C. O., & Rubiolo, P. (2008). Group-type and fingerprint analysis of roasted food matrices (coffee and hazelnut samples) by comprehensive two-dimensional gas chromatography. *Journal of Agricultural and Food Chemistry, 56*(17), 7655-7666.

Costa, P., Velasco, C. V., Loureiro, J. M., & Rodrigues, A. E. (2016). Effect of cosmetic matrices on the release and odour profiles of the supercritical CO2 extract of Origanum majorana L. *International Journal of Cosmetic Science, 38*(4), 364-374.

Costa, R., Fanali, C., Pennazza, G., Tedone, L., Dugo, L., Santonico, M., Sciarrone, D., Cacciola, F., Cucchiarini, L., Dachà, M., & Mondello, L. (2015). Screening of volatile compounds composition of white truffle during storage by GCxGC-(FID/MS) and gas sensor array analyses. *LWT - Food Science and Technology, 60*(2), 905-913.

Cullere, L., Ferreira, V., Venturini, M. E., Marco, P., & Blanco, D. (2013). Chemical and sensory effects of the freezing process on the aroma profile of black truffles (Tuber melanosporum). *Food chemistry, 136*(2), 518-525.

Elmore, J. S., Warren, H. E., Mottram, D. S., Scollan, N. D., Enser, M., Richardson, R. I., & Wood, J. D. (2004). A comparison of the aroma volatiles and fatty acid compositions of grilled beef muscle from Aberdeen Angus and Holstein-Friesian steers fed diets based on silage or concentrates. *Meat Science, 68*(1), 27-33.

Fischer, J., Haas, T., Leppert, J., Lammers, P. S., Horner, G., Wuest, M., & Boeker, P. (2014). Fast and solvent-free quantitation of boar taint odorants in pig fat by stable isotope dilution analysis-dynamic headspace-thermal desorption-gas chromatography/time-of-flight mass spectrometry. *Food chemistry, 158*, 345-350.

Insausti, K., Beriain, M. J., Gorraiz, C., & Purroy, A. (2002). Volatile compounds of raw beef from 5 local Spanish cattle breeds stored under modified atmosphere. *Journal of Food Science, 67*(4), 1580-1589.

Kerler, J., & Grosch, W. (1996). Odorants contributing to warmed-over flavor (WOF) of refrigerated cooked beef. *Journal of Food Science, 61*(6), 1271-&.

King, M. F., Hamilton, B. L., Matthews, M. A., Rule, D. C., & Field, R. A. (1993). Isolation and identification of volatiles and condensable material in raw beef with supercritical carbon-dioxide extraction. *Journal of Agricultural and Food Chemistry, 41*(11), 1974-1981.

La Storia, A., Ferrocino, I., Torrieri, E., Di Monaco, R., Mauriello, G., Villani, F., & Ercolini, D. (2012). A combination of modified atmosphere and antimicrobial packaging to extend the shelf-life of beefsteaks stored at chill temperature. *International Journal of Food Microbiology, 158*(3), 186-194.

Machiels, D., & Istasse, L. (2003). Evaluation of two commercial solid-phase microextraction fibres for the analysis of target aroma compounds in cooked beef meat. *Talanta, 61*(4), 529-537.

Met, A., & Yesilcubuk, N. S. (2017). Comparison of Two Volatile Sampling Techniques Based on Different Loading Factors in Determination of Volatile Organic Compounds Released from Spoiled Raw Beef. *Food Analytical Methods, 10*(7), 2311-2324.

Mirnaghi, F. S., Mousavi, F., Rocha, S. M., & Pawliszyn, J. (2013). Automated determination of phenolic compounds in wine, berry, and grape samples using 96-blade solid phase microextraction system coupled with liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A, 1276*, 12-19.

Oliver-Pozo, C., Aparicio-Ruiz, R., Romero, I., & Garcia-Gonzalez, D. L. (2015). Analysis of Volatile Markers for Virgin Olive Oil Aroma Defects by SPME-GC/FID: Possible Sources of Incorrect Data. *Journal of Agricultural and Food Chemistry, 63*(48), 10477-10483.

Ouyang, G. F., & Pawliszyn, J. (2008). A critical review in calibration methods for solid-phase microextraction. *Analytica Chimica Acta, 627*(2), 184-197.

Panseri, S., Chiesa, L. M., Biondi, P. A., Rusconi, M., Giacobbo, F., Padovani, E., & Mariani, M. (2015). Irradiated ground beef patties: Dose and dose-age estimation by volatile compounds measurement. *Food Control, 50*, 521-529.

Pawliszyn, J. (2009). *Handbook of solid phase microextraction*: Chemical Industry Press.

Perez, R. A., Rojo, M. D., Gonzalez, G., & De Lorenzo, C. (2008). Solid-Phase Microextraction for the Determination of Volatile Compounds in the Spoilage of Raw Ground Beef. *Journal of Aoac International, 91*(6), 1409-1415.

Pfalzgraf, A., Frigg, M., & Steinhart, H. (1995). Alpha-tocopherol contents and lipid oxidation in pork muscle and adipose-tissue during storage. *Journal of Agricultural and Food Chemistry, 43*(5), 1339-1342.

Resconi, V. C., Bueno, M., Escudero, A., Magalhaes, D., Ferreira, V., & Mar Campo, M. (2018). Ageing and retail display time in raw beef odour according to the degree of lipid oxidation. *Food chemistry, 242*, 288-300.

Resconi, V. C., Escudero, A., Beltran, J. A., Olleta, J. L., Sanudo, C., & Campo, M. D. (2012). Color, Lipid Oxidation, Sensory Quality, and Aroma Compounds of Beef Steaks Displayed under Different Levels of Oxygen in a Modified Atmosphere Package. *Journal of Food Science, 77*(1), S10-S18.

Rivas-Canedo, A., Juez-Ojeda, C., Nunez, M., & Fernandez-Garcia, E. (2011a). Effects of high-pressure processing on the volatile compounds of sliced cooked pork shoulder during refrigerated storage. *Food chemistry, 124*(3), 749-758.

Rivas-Canedo, A., Juez-Ojeda, C., Nunez, M., & Fernandez-Garcia, E. (2011b). Volatile compounds in ground beef subjected to high pressure processing: A comparison of dynamic headspace and solid-phase microextraction. *Food chemistry, 124*(3), 1201-1207.

Rivas-Canedo, A., Juez-Ojeda, C., Nunez, M., & Fernandez-Garcia, E. (2012). Volatile compounds in low-acid fermented sausage "espetec" and sliced cooked pork shoulder subjected to high pressure processing. A comparison of dynamic headspace and solid-phase microextraction. *Food chemistry, 132*(1), 18-26.

Rochat, S., & Chaintreau, A. (2005). Carbonyl odorants contributing to the in-oven roast beef top note. *Journal of Agricultural and Food Chemistry, 53*(24), 9578-9585.

Rota, V., & Schieberle, P. (2005). Changes in Key Odorants of Sheep Meat Induced by Cooking. In *Food Lipids*, vol. 920 (pp. 73-83): American Chemical Society.

Saraiva, C., Oliveira, I., Silva, J. A., Martins, C., Ventanas, J., & Garcia, C. (2015). Implementation of multivariate techniques for the selection of volatile compounds as indicators of sensory quality of raw beef. *Journal of Food Science and Technology-Mysore, 52*(6), 3887-3898.

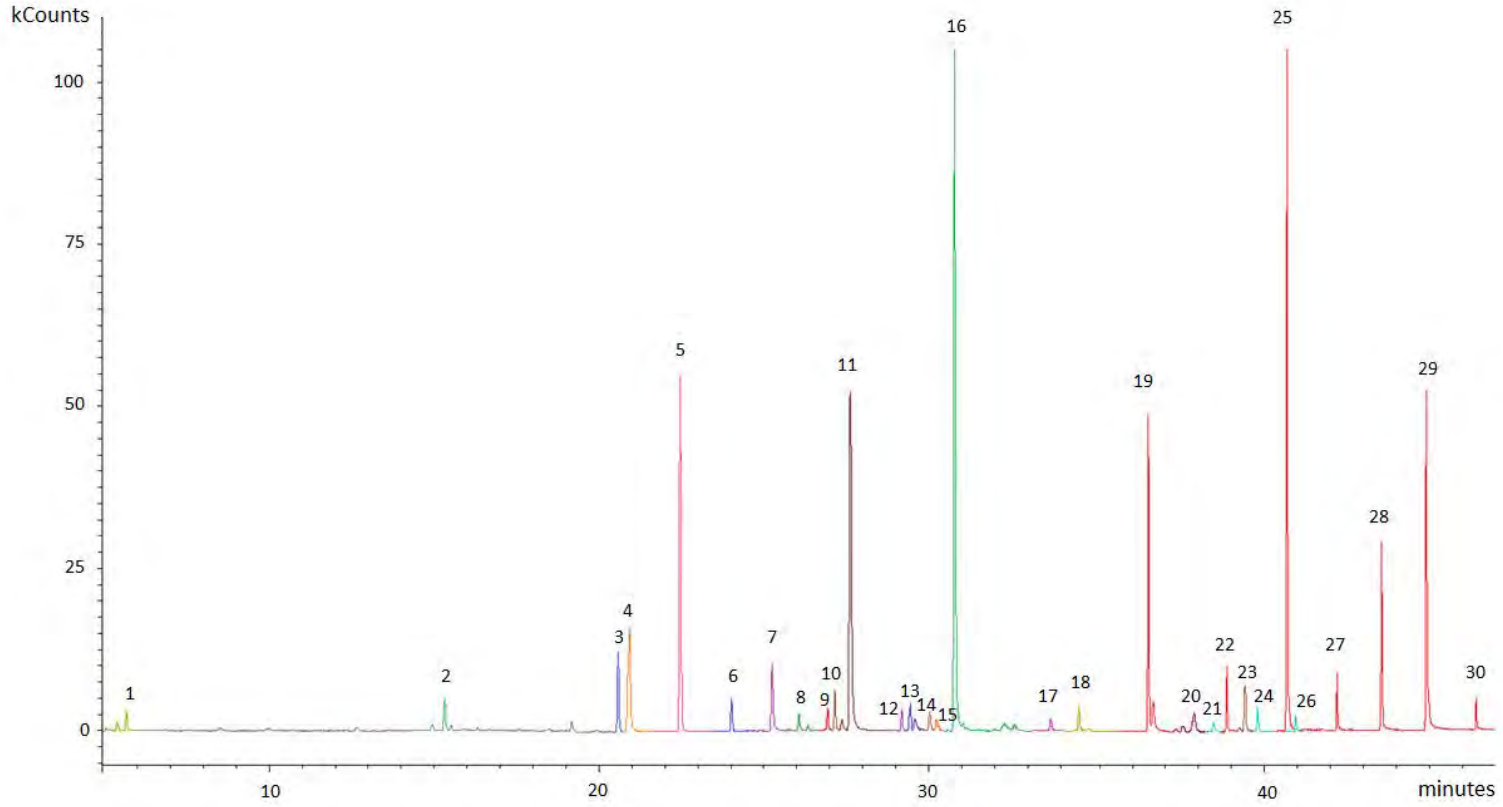
Schindler, S., Krings, U., Berger, R. G., & Orlien, V. (2010). Aroma development in high pressure treated beef and chicken meat compared to raw and heat treated. *Meat Science, 86*(2), 317-323.

Song, S. Q., Zhang, X. M., Hayat, K., Liu, P., Jia, C. S., Xia, S. Q., Xiao, Z. B., Tian, H. X., & Niu, Y. W. (2011). Formation of the beef flavour precursors and their correlation with chemical parameters during the controlled thermal oxidation of tallow. *Food chemistry, 124*(1), 203-209.

Souza-Silva, E. A., Gionfriddo, E., & Pawliszyn, J. (2015). A critical review of the state of the art of solid-phase microextraction of complex matrices II. Food analysis. *Trac-Trends in Analytical Chemistry, 71*, 236-248.

Figures

(COLOURED) Figure 1. Processed MS chromatogram formed from 16 ion chromatograms of target compounds obtained from a low oxidative group sample of 7 days of display with the proposed procedure. Compounds identification and m/z are shown in Table 1. Different m/z have different colours.



(COLOURED) Figure 2. (*E*)-2-nonenal control chart. DCA: 2,6-dichloroanisole; C.S.: control solution

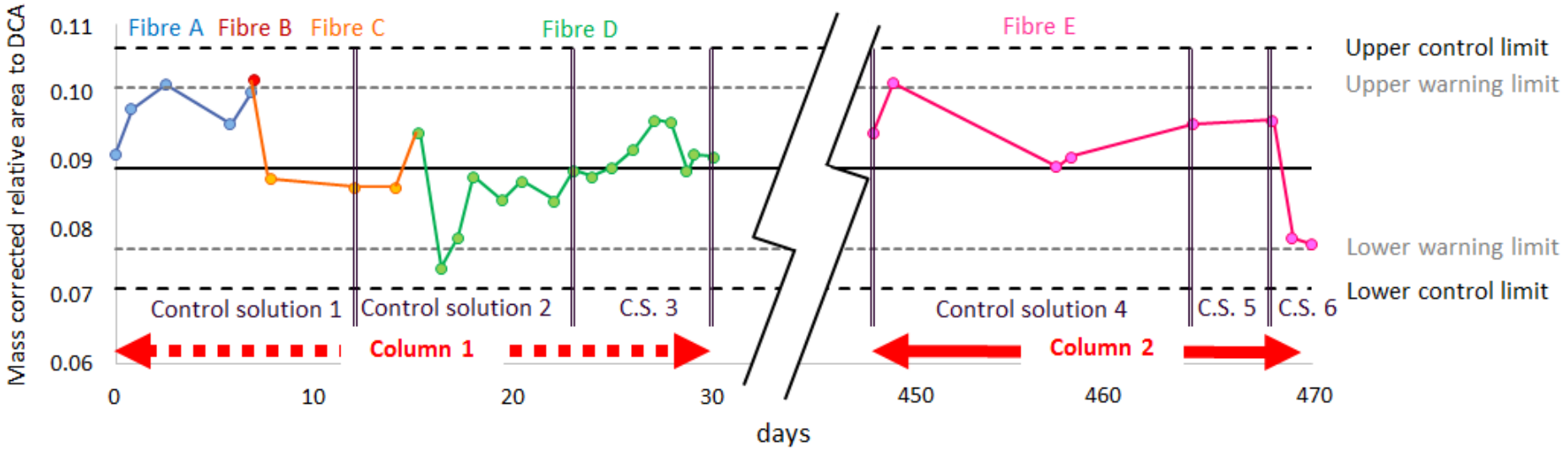
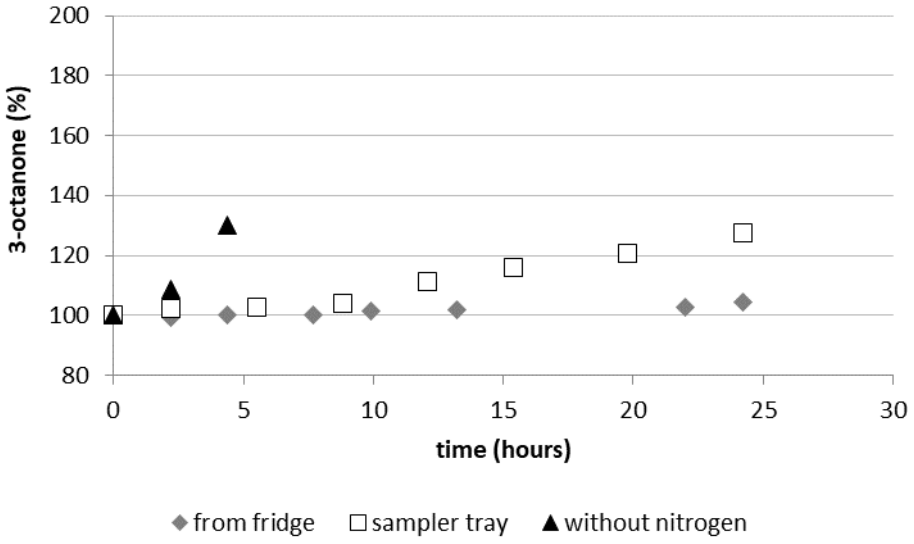
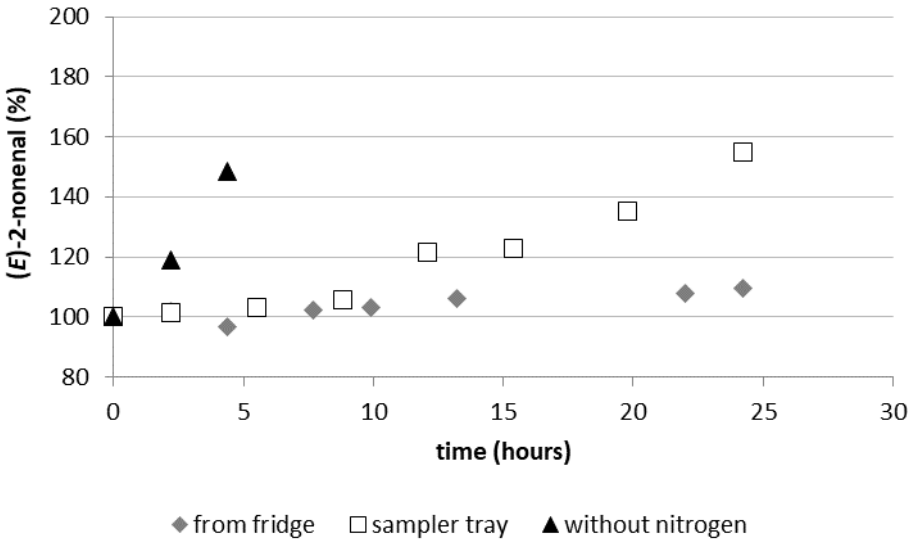


Figure 3. Effect of the time in the relative percentage of oxidation related compounds in the analysis of vials kept in different conditions. a) 3-octanone, b) (*E*)-2-nonenal, c) butanoic acid

a)



b)



c)

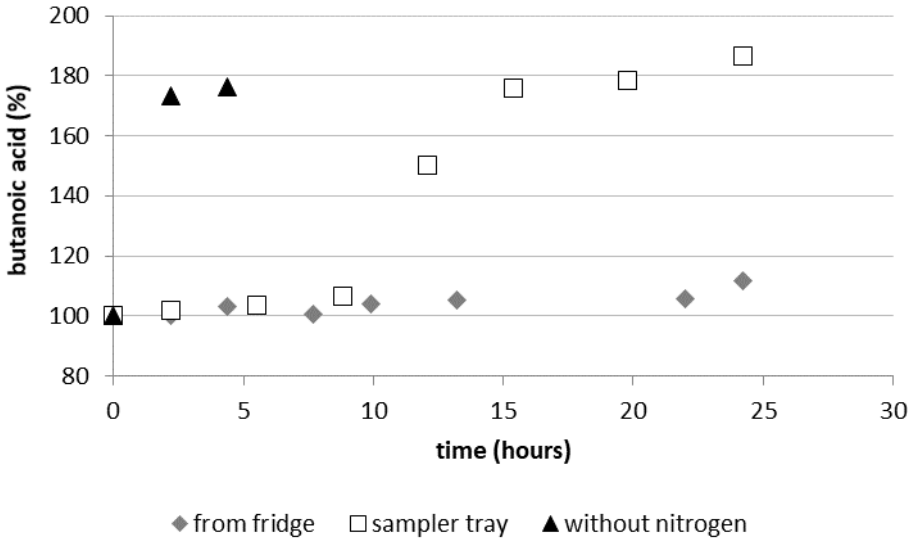


Figure 4. PCA plot with data from 48 samples from day 9 of display of the three different oxidative groups: low (circles), medium, (squares) and high (triangles)

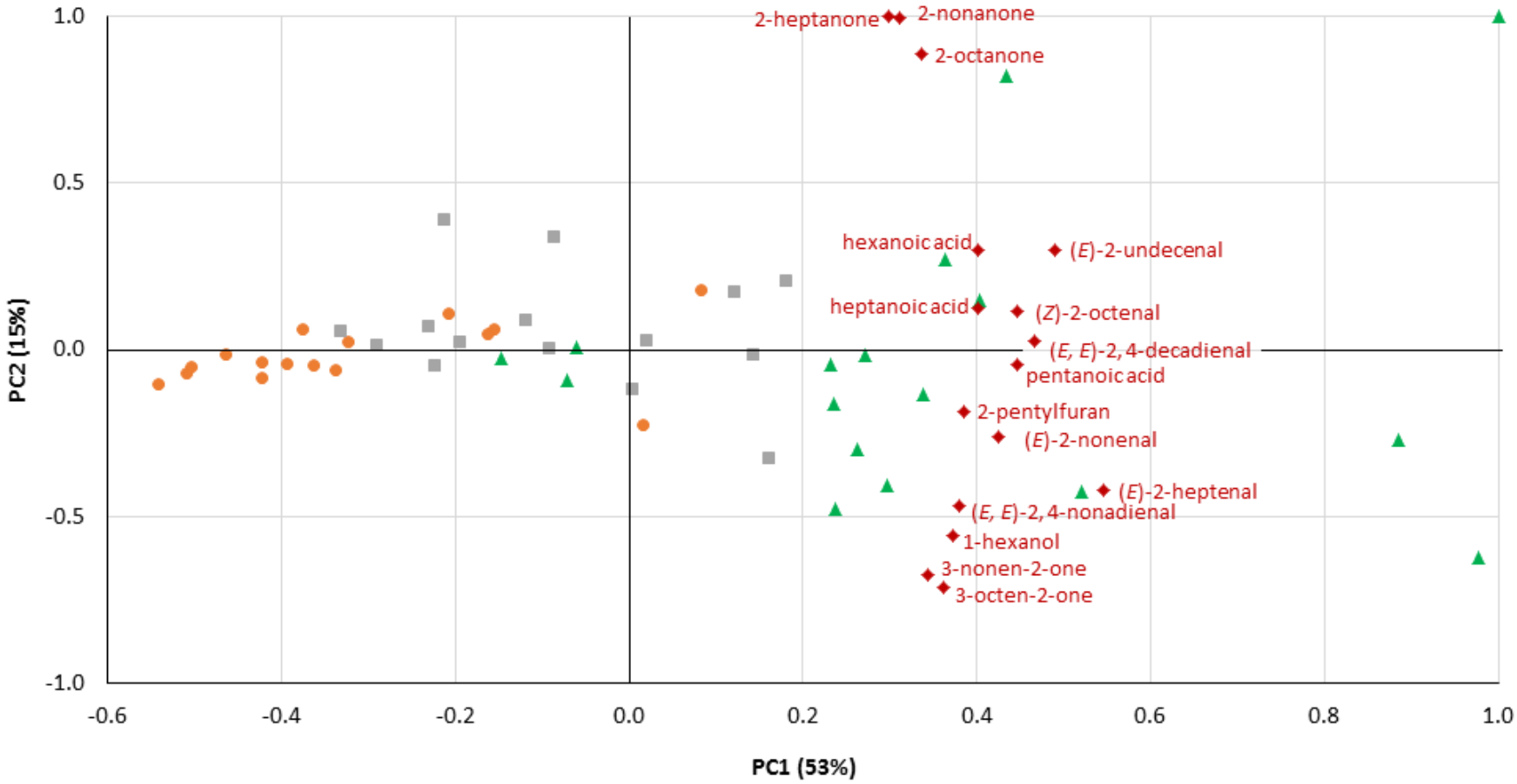


Table 1. Masses of the ions selected for the determination of the analytes considered in the study and their retention data in a DB-WAX column.

Chomatographic order	Analyte	m/z	Experimental LRI	Referenced LRI	Meat reference
	Acids				
19	butanoic acid[a]	60	1632	1626	1
22	pentanoic acid[a]	60	1744	1752	2
25	hexanoic acid[a]	60	1851	1861	3
27	heptanoic acid[a]	60	1975	1975	2
28	octanoic acid[a]	60	2051	2076	3
29	nonanoic acid[a]	60	2116	2131	4
30	decanoic acid[b]	60	2200		
	Saturated aldehydes				
2	hexanal[a]	57	1052	1071	5
3	heptanal[a]	71	1188	1193	6
13	nonanal[a]	98+120	1402	1399	6
20	phenylacetaldehyde	91+120	1681	1689	7
	Alkenals				
10	(*E*)-2-heptenal[a]	83	1344	1362	7
14	(*Z*)-2-octenal[c]	83	1420	1402	5
18	(*E*)-2-nonenal[a]	93	1562	1556	8
23	(*E*)-2-undecenal[a]	83	1781	1789	7
21	(*E, E*)-2,4-nonadienal[a]	81	1741	1741	7
24	(*E, Z*)-2,4-decadienal[c]	81	1799	1778	6
26	(*E, E*)-2,4-decadienal[a]	81	1854	1850	7
	Saturated ketones				
1	2-butanone[b]	72	668		
4	2-heptanone[a]	99	1192	1195	9
7	2-octanone[a]	58	1293	1289	9
6	3-octanone[a]	99	1265	1260	9
9	2,3-octanedione[a]	142+125	1332	1328	9
12	2-nonanone[a]	58	1400	1408	10
	Unsaturated ketones				
8	1-octen-3-one[a]	70	1311	1315	1
15	3-octen-2-one[b]	111	1427		
17	3-nonen-2-one[a]	125	1535	1516	8
	Other compounds				
11	1-hexanol[a]	69	1359	1355	6
16	1-octen-3-ol[a]	57	1449	1451	6
5	2-pentylfuran[a]	138	1235	1240	3
	Control solution IS				
	methyl 2-methylbutyrate	88	1000		
	2,6-dichloroanisole	176	1749		

LRI: linear retention index
IS: Internal Standard
[a] Identification based on the similarity observed chromatographic retention in DB-WAX column, mass spectrometric data and confirmed by injection of the pure reference standard.
[b] Identification based on mass spectrometric data and confirmed by injection of the pure reference standard.
[c] Identification based on the similarity observed chromatographic retention in DB-WAX column, mass spectrometric data and found in the standards from their isomers.
Reference for compounds previously identified in meat: 1) (Kerler & Grosch, 1996), 2) (Rivas-Canedo, Juez-Ojeda, Nunez, & Fernandez-Garcia, 2011a), 3) (Rivas-Canedo, Juez-Ojeda, Nunez, & Fernandez-Garcia, 2011b), 4) (Almela, Jose Jordan, Martinez, Antonio Sotomayor, Bedia, & Banon, 2010), 5) (Schindler, Krings, Berger, & Orlien, 2010), 6) (Song, Zhang, Hayat, Liu, Jia, Xia, et al., 2011), 7) (Rochat & Chaintreau, 2005), 8) (V. C. Resconi, Escudero, Beltran, Olleta, Sanudo, & Campo, 2012), 9) (Elmore, Warren, Mottram, Scollan, Enser, Richardson, et al., 2004), 10) (Rivas-Canedo, Juez-Ojeda, Nunez, & Fernandez-Garcia, 2012)

Table 2. Method repeatability (*n*=5) and reproducibility (*n*=4) in terms of relative standard deviation.

Analyte	Repeatability RSD (%)	Reproducibility RSD (%)
Acids		
butanoic acid	16	30
pentanoic acid	16	19
hexanoic acid	13	15
heptanoic acid	13	23
octanoic acid	8	20
nonanoic acid	9	15
decanoic acid	13	28
Saturated aldehydes		
hexanal	14	18
heptanal	12	13
nonanal	11	14
phenylacetaldehyde	16	24
Alkenals		
(*E*)-2-heptenal	11	12
(*Z*)-2-octenal	11	14
(*E*)-2-nonenal	14	24
(*E*)-2-undecenal	3	15
(*E, E*)-2,4-nonadienal	7	13
(*E, Z*)-2,4-decadienal	17	24
(*E, E*)-2,4-decadienal	15	17
Saturated ketones		
2-butanone	7	12
2-heptanone	5	12
2-octanone	4	12
3-octanone	8	13
2,3-octanedione	7	13
2-nonanone	5	6
Unsaturated ketones		
1-octen-3-one	6	9
3-octen-2-one	10	11
3-nonen-2-one	5	9
Other compounds		
1-hexanol	5	6
1-octen-3-ol	3	7
2-pentylfuran	9	14

RSD: relative standard deviation

- A HS-SPME-GC-MS method for the reliable quantification of 30 raw beef volatiles was developed.
- The inclusion of a control solution overcomes several challenges associated with the analysis of solid samples.
- Method variability is sufficient for studying the volatile profile from samples with different lipid oxidation degrees.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: