RAPID DETECTION OF LIPID OXIDATION IN BEEF MUSCLE PACKED UNDER MODIFIED ATMOSPHERE BY MEASURING VOLATILE ORGANIC COMPOUNDS USING SIFT-MS

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Abstract

The objective of this work was to evaluate the use of a direct analysis technique (SIFT-MS) to measure the lipid oxidation process in beef meat packed under high oxygen atmosphere and compare it to conventional techniques such as gas chromatography-mass spectrometry analysis and TBARS values. Meat samples from two suppliers were selected and packaged under the same atmosphere conditions. The fatty acid content, the physicochemical (TBARS and volatile compounds) and sensory parameters were measured. The samples from supplier 2 had a highest content of PUFA and n6 fatty acids that was related with a highest oxidation during storage. SIFT-MS and SPME-GC-MS detected a significant increase for most of the volatiles compounds analyzed during storage especially, in aldehyde compounds. High correlation coefficients between TBARS values and linear aldehydes (C3 to C7) measured by both techniques were obtained and this indicates that SIFT-MS can be used to monitor lipid oxidation changes.

Keywords: SIFT-MS, beef, oxidation, volatile organic compounds, modified atmosphere packaging

1. Introduction

Nowadays, the use of Modified Atmosphere Packaging (MAP) for retail meat is one of the main trends of the market. The meat appearance is a very important factor for consumers who expect the bright red colour as an index of freshness (Zakrys-Waliwander, O'Sullivan, Walsh, Allen, Kerry, 2011). However the use of high oxygen atmosphere to favour the generation of the bright red colour, promotes oxidative changes in meat. This process negatively affects meat quality producing the generation of off-flavours (Campo, Nute, Hughes, Enser, Wood, Richardson, 2006), colour deterioration (Zakrys, O'Sullivan, Allen, Kerry, 2009) and a decrease in tenderness (Lund, Lametsch, Hviid, Jensen, Skibsted, 2007, Kim, Huff-Lonergan, Sebranek, Lonergan, 2010, Clausen, Jakobsen, Ertbjerg, Madsen, 2009, Lagerstedt, Lundstrom, Lindahl, 2011). This decrease in tenderness has been attributed to the oxidation of muscle proteins (Rowe, Maddock, Lonergan, Huff-Lonergan, 2004) although the effect of high oxygen packaging on beef tenderness has not been completely elucidated (Zakrys-Waliwander, O'Sullivan, O´Neill, Kerry, 2012). In addition, during the shelf life of modified atmosphere meat the dominated bacteria are lactic acid bacteria (LAB) that are also responsible for the generation of off-flavours and off-odours (Zakrys-Waliwander et al., 2012).

It is well known that beef quality depends on many factors such as breed, sex, animal age and feeding regimen (Raes, Balcaen, Dirinck, De Winne, Claeys, Demeyer, De Smet, 2003). All these factors affect fatty acid composition of bovine tissue (Aldai, Murray, Olivan, Martinez, Troy, Osoro, Najera, 2006) being beef meat susceptible to oxidation. The lipid fraction with the highest susceptibility to oxidation and development of rancid flavours due to its high number of double bonds is the phospholipid fraction although, its percentage in bovine muscle is lower than the neutral lipid fraction (Gokalp, Ockerman, Plimpton, Harper, 1983, Campo et al., 2006).

Generally, lipid oxidation is measured by chemical methods (peroxides, cholesteroloxides, hexanal, volatile compounds) because they are objective although these chemical methods are time consuming. The one most widely used is the analysis of malonaldehyde by the thiobarbituric acid reaction (TBARS) or the measurement of linear aldehydes by GC analysis

(Stangelo, Vercellotti, Legendre, Vinnett, Kuan, James, Dupuy, 1987). Other methods are based on consumer panels but they have the limitation to be subjective. However, the main interest is to relate sensory perception to chemical measurements in order to determine the limit of rancidity detected by consumers in beef meat (Campo et al., 2006). Moreover, all these analyses, chemical and sensory, are time consuming and there is not a fast reliable technique to measure the lipid oxidation and development of off-flavours in MAP beef meat.

Recently, new techniques focused on the direct analysis of volatile compound in air using different ionization techniques have been developed. This is the case of selected ion flow tube mass spectrometry (SIFT-MS; Španěl and Smith, 1999; Smith and Spanel, 2005) that it is based on the chemical ionization of a gas sample using specific, selected precursor (reagent) positive ions. SIFT-MS allows the real time quantification of a volatile compound in humid air without external calibration. The absolute concentrations are calculated from the ratios of the count rates of the product analyte-derived ions to those of the precursor whilst taking into account known values of the reaction rate coefficients, reaction time and the influence of diffusion and mass discrimination (Smith, Pysanenko, Španěl, 2009).

SIFT-MS has been previously applied to different foods although few studies have been focused on the measurement of food quality (in olive oil, Davis, McEwan, 2007, Davis Senthilmohan, Wilson, McEwan, 2005) while other studies reported the quantification of aldehydes from malt (De Clippeleer, Opstaele, Francis, Cooman, Aerts, 2010). However, there are no reports about its usefulness in retail meat whereas other ionization technique such as proton transfer reaction mass spectrometry (PTR-MS) was used for the detection of meat spoilage (Mayr, Margesin, Klingsbichel, Hartungen, Jenewein, Schinner, Maerk, 2003). This study proved the relationship between several masses (63, 91, and others) and bacterial counts in meat packaged in air and vacuum and finally, they proposed the use of PTR-MS for online measurements of contaminated meats. However, none of these studies have studied the effect of meat composition on the release of volatile compounds.

In order to study the application of this new direct analysis technique (SIFT-MS) and its possible applicability to determine the oxidative stability of MAP beef meat in a real fast manner

92 we selected meat from two suppliers to obtain meat with different susceptibility to oxidation.
93 Therefore, the objective of this work was to obtain an objective measurement of the lipid
94 oxidation process in beef meat packed under high oxygen atmosphere using SIFT-MS.
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96 **2. Materials and Methods**
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98 *2.1 Beef samples and Packaging*
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100 Beef steaks, muscle *longissimus dorsi* (LD) were obtained in collaboration with a local meat
101 processor from two different suppliers. Both suppliers produced beef cattle of 11 to 12 months
102 old but from different breeds Supplier 1 was Charolais while supplier 2 was Simmental. All the
103 animals were fed with grass silage and concentrate. The medium final weight of carcasses at
104 slaughter was 237.5 and 249.7 kg for each supplier respectively. For each type of supplier
105 three animals were selected and their LD muscle was sliced. Then, the muscle of each animal
106 was cut into uniform 1.5 cm thick steaks. Steaks were packed under MAP (20%, CO_2 and 80%
107 O_2) in polystyrene/EVOH/polyethylene trays, heat sealed with laminated barrier film and stored
108 at 4°C. From each animal, steaks were sampled at 0, 2, 5, 8 and 12 days of storage. One steak
109 was divided in portions to perform the chemical analyses: lipid profile (only at 0 day), lipid
110 oxidation (TBARS), aroma by GC-MS and aroma by SIFT-MS (at 0, 2, 5, 8 and 12 d). In
111 addition, another steak was cooked and grilled at 95°C for 2.5 min each side until reached an
112 internal temperature of 74°C measured by a puncture digital thermometer and used for aroma
113 analysis by GC-MS and SIFT-MS (at 0, 5 and 8d). All the samples were vacuum packaged and
114 stored frozen at -80°C until analysis. Finally, a sensory analysis on the cooked steaks was
115 performed directly at 0, 5 and 8 days.
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2.2 Lipid oxidation

The lipid oxidation in steaks was determined using the thiobarbituric acid reactive substances (TBARS) method, as described by Witte, Krauze, Bailey (1970), using tricloroacetic acid instead of perchloric acid as solvent. The results were expressed as mg malonaldehyde (MDA) per kg of meat. The lipid oxidation determinations were replicated three times and the results expressed as the mean of the replicates.

2.3 Extraction of total fatty acids and lipid analysis

Total lipids were extracted from 10 g of minced steak according to the method of Folch, Lees and Stanlye (1957), using dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1) as solvent due to its lower toxicity. The extracts were dried in a rotating vacuum evaporator and weighed to determine the total lipid quantity. Total fatty acids were methylated according to the method of Berry, Cevallos, Wade, (1965). Analysis was carried out in a Fisons 8160 gas chromatograph (GC) equipped with a flame ionisation detector as described Olivares, Navarro, Flores (2011) and using Heneicosanoic acid (C21:0) as the internal standard. The individual FAME were identified by comparing their retention times with those of standard fatty acid methyl esters (Supelco). For quantification, the response factors of the standard FAME with respect to the internal standard were calculated. FA content was expressed as a percentage of the amount of total methyl esters. The results were expressed as the mean of three replicates in meat from each supplier.

2.4 Sensory analysis

The acceptability of meat samples at 0 days and after storage for 5 and 8 days at 4°C was evaluated by 50 consumer panellists. Testing was carried out in a sensory laboratory equipped with individual booths (ISO 8589, 1988). The meat was evaluated after grilled at 95°C for 2.5

145 min each side until reached an internal temperature of 74°C measured by a puncture digital
146 thermometer. Uniforms cubes were cut wrapped in aluminium foil and kept warm until sensory
147 analysis was done. The cubes were served at room temperature on three-digit coded white
148 plastic dishes. Water and unsalted toasts were provided to cleanse the palate between
149 samples. The consumers were asked to evaluate each beef sample based on aroma, taste,
150 hardness, juiciness, overall acceptability and appearance using a 9-point hedonic scale. The
151 analysis was done in three different sessions at each storage time.
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153 *2.5 SPME-GC-MS analysis*
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155 The analysis of volatile compounds in the headspace (HS) of beef sample was done as
156 described Olivares et al (2011). Beef meat was minced with liquid nitrogen and 0.75 mg of
157 antioxidant (butylated hydroxytoluene, BHT) was added. Then five g of the minced beef meat
158 was weighted into a 10 mL headspace vial. The vial was equilibrated for 1 h in a thermoblock
159 (J.P., Selecta, Barcelona, Spain) at 37 ºC. The volatile compounds were extracted by solid
160 phase micro-extraction (SPME) using a 85 µm carboxen/polydimethylsiloxane StableFlex fibre
161 (CAR/PDMS SF, Supelco, Bellefonte, Pennsylvania, USA) for 3 h while maintaining the sample
162 at 37 ºC. The fibre was then injected in the split-less mode in a gas chromatograph (HP 7890A)
163 equipped with a HP 5975C mass selective detector (Hewlett Packard, Palo Alto, CA). The
164 released compounds were separated using a DB-624 capillary column (J & W Scientific, Agilent
165 Technologies, USA) and identified by comparison to the mass spectra from the (NIST 05)
166 library database, to linear retention index (Kovats, 1965) and using authentic standards. The
167 volatile compounds were analyzed in SIM mode and a selected m/z ion of each compound was
168 used for quantification. The headspace of each beef sample was analyzed in duplicate.
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170 *2.6 SIFT-MS analysis*
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The quantification of the volatile compounds by SIFT-MS in the headspace of beef samples was carried out using the method previously described in our paper on study of volatiles released from fermented sausage (Olivares, Dryahina, Navarro, Flores, Smith, Španěl, 2010, Olivares, Dryahina, Navarro, Smith, Spanel, Flores, 2011) using the identical SIFT-MS *Profile 3* instrument (Instrument Science Limited, UK) with the flow tube diameter of 1 cm and reaction length of 4 cm. H_3O^+, NO^+ and O_2^+ ions were used as precursors for chemical ionisation and their count rates were in the range from 100000 to 1000000 counts/second. Flow tube temperature was 26 ºC, flow tube pressure was 1.0 Torr. The multiple ion monitoring (MIM) mode was used to quantify specific volatile compounds (Španěl, Dryahina, and Smith, 2006; Spanel and Smith, 2007). In this mode, the analytical mass spectrometer is rapidly switched between selected m/z values of both the precursor ions and the characteristic product ions. The actual m/z values used in the present study are listed for almost all compounds in Olivares et al (2010) except for four compounds. H_3O+ was used as precursor ion for the analysis of acetaldehyde and butyric acid and the products ions selected were 45+81 and 89+107+125, respectively. In addition, 2-butenal and 2-hexenal were analysed using as precursor ion NO^+ and the product ions were 69 and 97+128, respectively. The known rate coefficients for the analytical reactions were then used to quantify the absolute HS concentrations of the compounds using the standard SIFT-MS data analysis software and the general method of quantification (Španěl, Dryahina, and Smith, 2006). Ionic diffusion and mass discrimination was corrected by the SIFT-MS software according to procedure described in Smith et al. (2009). The absolute quantification was continuously verified by analyses of absolute humidity.

For each measurement, 5 g of beef meat was weighted into a 15 mL headspace vial, together with 0.75 mg of butylated hydroxytoluene (BHT) used as antioxidant. The emitted volatiles were allowed to develop in the HS of the sealed vial (initially purged with laboratory air) at 37 ºC for 1 hour. The air/volatile compounds were sampled directly by piercing the septum by a stainless steel needle connected directly to the SIFT-MS sampling line. The sample entered the helium carrier gas via a heated (70°C) capillary tube at a measured rate of 0.45 Torr L/s. A second needle pierced through the septum was used to maintain the pressure in the vial at atmospheric

pressure by introducing laboratory air at a rate that balances the small loss rate due to the sampling into the SIFT-MS instrument. Background (laboratory air) concentrations of all the volatile compounds included in the analysis were routinely recorded before and after the analysis of each sample. Data for each precursor ion were collected and integrated for a period of 200 seconds and the mean values over this sampling time were recorded. The results were then expressed in parts-per-billion by volume of the headspace, ppbv (nL of volatile compound per L of air). The headspace of the beef meat was analyzed in duplicate. The measuring order of the samples was randomised.

2.7 Statistical analysis

The effect of the different meat supplier and storage time on the HS volatile compounds concentration obtained by both techniques was assessed using analysis of variance (ANOVA). Pearson correlation analysis was performed to correlate the results obtained by SIFT-MS and SPME-GC-MS analyses and also the results were correlated to the oxidative status of beef samples (TBARS values). The statistical software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain) package was used for these analyses. Furthermore, principal component analysis (PCA) was used to find the relationships among beef samples storage at different times and the parameters related to lipid composition (SAT, MUFA, PUFA, n-6 and n-3 fatty acids), oxidation values (TBARS) and volatile compounds from SIFT-MS analysis.

3. Results and discussion

3.1 Beef lipid composition, lipid oxidation and sensory acceptability during refrigerated storage.

The total intramuscular fatty acid content of meat from both suppliers was not significantly different and was 2.44 and 2.45 %, respectively. However, they were different in total fatty acid composition (table 1). The beef from the first supplier showed a significant highest proportion of

saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) although the PUFA content was not significant. However, meat from supplier 1 had a lowest significant n-6 content and highest content of n-3 fatty acids. Probably, breed is the reason for differences in fatty acid composition as both suppliers used the same feeding regimen (Wood, Enser, Fisher, Nute, Richardson, Sheard, 1999). The highest n-3 fatty acid content of meat from supplier 1 resulted in a lower n-6/n-3 ratio, with values similar to other studies obtained with lean beef breeds (Raes et al., 2003). The actual nutritional guidelines for meat consumption recommend a n-6/n-3 ratio to be 5 or lower (Raes et al., 2003) but only supplier 1 fulfil this nutritional guideline.

The oxidative stability of the beef samples during refrigerated storage in high oxygen MAP packages was studied by measuring the TBARS values and the results are shown in figure 1. The oxidation values of beef steaks at day 0 of display were very low but the oxidative stability decreased during time as observed by an increase in TBARS values in meat from both suppliers. However, meat from supplier 2 showed the lowest oxidative stability as seen by the highest TBARS values during all times of storage. The high increase in lipid oxidation values in beef meat packaged under high oxygen atmospheres during display has been reported by many authors (Kim et al., 2010, Clausen et al., 2009). The lowest oxidative stability of beef from supplier 2 can be due to the PUFA content although it was not significantly different between the two suppliers. However the n-6 content was significantly highest in meat from supplier 2 while n-3 and CLA contents were the highest in meat from supplier 1. The highest content of n-6 total FAME in meat from supplier 2 can be responsible of the lowest oxidative stability as this n-6 content represents almost 80% of the total PUFA content.

Different authors have tried to predict the TBARS values based on PUFA composition. In 1999 Elmore et al., suggested that the autoxidation of the lipid fraction is initiated more readily by the presence of n-3 fatty acids but once the free radical reaction is started, the next reactions are less dependent on the nature of the unsaturated fatty acid and the autoxidation is due to the breakdown of the most abundant oleic and linoleic acids. On the other hand, Insausti et al., (2004) did not find this relationship when they studied the oxidative stability of different breeds

storage in MAP. These authors used two fatty acids (linolenic and linoleic acids) to predict the TBAR value however they obtained low correlation coefficients, although the equation obtained indicate the relationship between unsaturated fatty acids and TBAR values.

Taken into account the high TBARS values obtained at 12 d of storage for supplier 2, the sensory analysis was only performed at 0, 5 and 8 d of storage. The sensory analysis performed in cooked beef steaks showed also differences not only at different times of storage but also between the two suppliers (table 2). Beef from supplier 1 had the highest acceptance in aroma, taste and texture after 5 and 8 days of storage. This fact affected the acceptability of the meat as the consumer panel showed the highest acceptance for the meat from supplier 1 at all the different times analyzed. The highest oxidative rancidity detected in meat from supplier 2 could be responsible of an increase of off-flavour and therefore a lowest acceptability by consumers as it has also been reported in beef steaks storage in high oxygen MAP (Kim et al., 2010, Lund et al., 2007).

3.2 Analysis of volatile compounds during beef refrigerated storage using SIFT-MS and SPME-GC-MS.

The quantification of volatile compounds during refrigerated storage of beef meat in high oxygen atmospheres was performed with both techniques; the conventional SPME-GC-MS and the real time analytical technique SIFT-MS.

Twenty seven volatile compounds were analyzed by SIFT-MS (table 3) while using SPME-GC-MS the compounds analyzed were 21 compounds (table 4). It was not possible to analyze several compounds by SPME-GC-MS due to their low concentration in the HS or to the low affinity by the fiber resulting in low concentrations not detected by MS. The sample preparation conditions used for both techniques were the same however SPME-GC-MS required a total time of 5 h while SIFT-MS required only 1 hour for extraction and about 3 min for analysis using each precursor ion.

283 For SIFT-MS analysis, different precursor ions were used for each volatile compound to select
284 the appropriate product ions that allows the quantification of the volatile compound without
285 other overlapping compounds (Olivares et al., 2010). The selection of the conditions was made
286 based on previous work done on another meat product, fermented sausage (Olivares et al.,
287 2010, Olivares, Dryahina, et al., 2011). The analysis of the compounds by SPME-GC-MS was
288 optimized and the ions used for the SIM method are indicated in table 4.
289 The results of the quantification using SIFT-MS in raw aged beef from the two suppliers are
290 shown in table 3. The analysis indicated a significant increase for all the volatiles compounds in
291 samples from both suppliers during refrigerated storage except for 2-hexenal, ethyl acetate,
292 hydrogen sulphide, methanethiol and the alcohols methanol, ethanol and 1-propanol. Moreover
293 there was a significant effect of the supplier as observed by significant differences between
294 both suppliers at all the times of refrigerated storage except for butanal, 2-pentenal,
295 methanethiol and ethanol. The remarkable effect was observed by a highest significant
296 concentration of the volatile compounds observed in samples from supplier 2 that could be
297 related to the highest lipid oxidation values observed in these samples (figure 1). Therefore, the
298 results shown can be used as an index of the storage process of beef in high oxygen
299 atmospheres in order to relate them to sensory acceptability (Kim et al., 2010). In addition, the
300 same samples were also subjected to the conventional analysis by SPME-GC-MS and the
301 results shown in table 4. A significant increase of concentrations of almost all compounds
302 except for butyric acid and carbon disulfide was observed for samples from both suppliers.
303 Some reduction in concentrations of dimethyl sulphide and acetone was detected during
304 refrigerated storage (table 4). In addition, there were significant differences between both
305 suppliers for all the compounds except for propanal, acetic and butyric acids, dimethyl sulphide
306 and ethanol. It was remarkable that in comparison to SIFT-MS the differences observed during
307 storage times were less appreciated by SPME-GC-MS, in this case the differences were
308 observed at the end of the process (12 d, table 4) while by SIFT-MS significant differences
309 were detected since the day 8^{th} of storage mainly for supplier 2 that showed a highest increase
310 (table 3). This effect could be due to a higher standard deviation observed in SPME-GC-MS

311 due to the handling of the sample. Therefore, SIFT-MS was able to detect significant
312 differences at 8 d of storage on the following volatile compounds: acetaldehyde, propanal, 2-
313 pentenal, 2-heptenal, butyric acid, dimethyl sulphide and acetone. This fact is very important
314 from the sensory point of view because, as observed in figure 1, at 8 d of storage the samples
315 from supplier 2 reach the value of TBARS: 2 mg MDA/kg, that has been considered the limit
316 point from where rancid flavour overcome beef flavour and the maximum level for a positive
317 beef sensory perception (Campo et al., 2006).
318 A Pearson correlation analysis was done to determine if both techniques were able to detect
319 the same differences. The correlation between both techniques (SPME-GC-MS and SIFT-MS)
320 in raw aged beef (aged for 12 d) in each of the measured compounds were calculated. Also the
321 same correlations were calculated for the measurements done in cooked aged beef (aged for 8
322 d). In raw aged beef, significant correlations were obtained between both techniques for all the
323 volatile compounds except for butyric acid (data not shown). On the other hand, in cooked beef
324 meat only hexanal showed a significant correlation when it was measured by both techniques.
325 Moreover, hexanal had a lower correlation coefficient ($r = 0.690$, $p < 0.002$) in cooked beef than
326 in raw beef ($r = 0.819$, $p < 0.0001$). This fact was probably due to the shorter refrigerated
327 storage period of cooked beef (8 d). So in raw beef, both techniques showed an increase in the
328 concentration of the two compounds during storage.
329 Due to the small time required by SIFT-MS, this technique can be useful for monitoring lipid
330 oxidation changes in retail meat packaged in high oxygen atmospheres. Conventionally,
331 TBARS values are used as an index of lipid oxidation in meat (Gandemer, 2002) and have
332 been correlated with consumer perception of lipid oxidation (Campo et al., 2006). In previous
333 works done in fermented sausages, it was observed high correlation coefficients between
334 TBARS values and linear aldehydes (C3 to C7) measured by both techniques, SIFT-MS and
335 SPME-GC-MS. In the present study shorter refrigerated storage times are applied in
336 comparison to a meat product such as dry fermented sausages although we also observed
337 significant positive correlations (data not shown). However, higher positive and significant
338 correlations were detected in the raw aged beef than in the cooked beef. In raw aged beef all

the aldehydes measured by SIFT-MS showed significant positive correlation ($r > 0.6$) with TBARS values as also happens with SPME-GC-MS except for acetaldehyde. This could be due to the low concentration of acetaldehyde extracted by the SPME fiber due to its low affinity for this compound.

The effect of storage time and supplier was studied by principal component analysis (PCA) to establish the relationships among fatty acid composition, lipid oxidation (TBARS values), and volatile compounds. Only the volatile compounds measured by SIFT-MS were introduced in the analysis to determine the possibility to use them as markers of the lipid oxidation process in a real fast manner.

Results from PCA applied to mean scores of the parameters are summarized in figure 2. The PCA showed that about 94.3% of the variability was explained by two first principal components. Principal component 1 (PC 1) was the most important variable in terms of differences among samples as it accounted for 71.88 % of the total variability. PC1 was positively related with refrigerated storage time and TBARS values including several of the aldehydes compounds. On the other hand, principal component 2 (PC2, 22.4%) was positively related to supplier 2, saturated fatty acids, PUFA and n-6 contents and volatiles such as 1-propanol and 2-hexenal. In contrast, PC2 was inversely correlated to supplier 1, MUFA and n-3 contents. In summary, PC1 differentiated the suppliers based on storage time while PC2 on fat composition. So, the presence of saturated (SFA), PUFA and n6 fatty acids in supplier 2 was related with the higher oxidation during storage in high oxygen packages. Finally, the measurement of aldehydes compounds by SIFT-MS can be used as markers of the lipid oxidation process in a real fast manner.

However, it is important to take into account the effect of the meat microbiota on volatile production. Ercolini, Ferrocino, Nasi, Ndagijimana, Vernocchi, Storia, Laghi, Mauriello, Guerzoni, Villani, (2011) indicated that the meat microbiota is highly affected by storage conditions and the microbial diversity is responsible for changes in the metabolites produced during meat storage. In addition, Ercolini et al., (2011) found a relation of MAP storage with the generation of acetoin and 1-octen-3-ol. In the present study we analyzed compounds derived

from chemical lipid oxidation such as aldehydes and few acids, sulfur, alcohol and ketone compounds although compounds such as acetoin and 1-octen-3-ol were not analyzed. Nevertheless, we did not observe a high generation of typically microbial compounds; methanethiol and ethanol, only butyric and acetic acids showed a significant increase with storage time as observed by SIFT-MS. Therefore, studies on meat volatile compounds will depend on the storage conditions and it is necessary to know the type of storage to select the compounds to be measured. Moreover, studies from Mayr et al., (2003) and Ercolini et al., (2011) did not take into account the effect of meat composition on volatile generation as we reported in the present study. In summary, there are many factors that should be taken into account to study the microbial and chemical spoilage of storage meat in order to develop an optimized method for meat quality control.

4. Conclusions

Two different meat samples were analyzed and showed a different oxidative behaviour during the refrigerated storage. One of the samples (supplier 2) had a highest content of PUFA and n6 fatty acids that was related with a highest oxidation during storage as observed by the highest TBARS values. SIFT-MS and SPME-GC-MS detected a significant increase for most of the volatiles compounds analyzed in both suppliers during refrigerated storage especially, in the content of aldehyde compounds. Also a highest significant concentration of aldehydes was observed in supplier 2. Moreover, SIFT-MS was able to detect differences earlier than SPME-GC-MS since the 8^{th} day of storage in raw meat while differences were hardly appreciated in cooked meat. Finally, high correlation coefficients between TBARS values and linear aldehydes (C3 to C7) measured by both techniques, SIFT-MS and SPME-GC-MS were obtained and this suppose that SIFT-MS can be used to monitor lipid oxidation changes as a fast measurement in retail meat packaged in high oxygen atmospheres.

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504 **Figure Legends**

505

506 Figure 1. Levels of TBARS (mg MDA/kg) during the refrigerated storage of raw beef meat from
507 different suppliers; supplier 1 (○) and supplier 2 (□). Symbols represent the mean and standard
508 error of the mean.

509

510 Figure 2. Loadings of the first two principal components (PC1-PC2) of the selected variables for
511 raw beef suppliers at different refrigerated storage times. The selected variables were the
512 volatile compounds (from SIFT-MS analysis), TBARS values (TBARS), fatty acid content:
513 saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids
514 (PUFA), n-6 PUFA fatty acids (n-6) and n-3 PUFA fatty acids (n-3). (■) suppliers and different
515 storage times, (●) instrumental variables.

516

Table 1. Fatty acid composition, as proportion of total FAME, of *longissimus dorsi* muscle depending on the supplier.

	Supplier 1		Supplier 2		
	mean	**sem**	**mean**	**sem**	**P**
C14:0	2.16	0.22	1.91	0.06	ns
C16:0	24.15	0.38	25.31	0.23	*
C18:0	13.72	0.75	18.73	0.18	***
C20:0	0.10	0.01	0.18	0.00	***
SFA	40.14	0.86	46.12	0.38	***
C16:1	4.12	0.27	2.94	0.05	***
C18:1	39.88	1.07	33.58	0.42	***
MUFA	44.00	1.25	36.52	0.43	***
C18:2 n-6	8.55	0.59	10.88	0.29	**
C18:3 n-3	0.47	0.01	0.29	0.01	***
C20:2 n-6	0.10	0.01	0.15	0.00	***
C20:3 n-6	0.80	0.06	0.76	0.03	ns
C20:4 n-6	2.97	0.21	3.36	0.14	ns
C22:4 n-6	0.42	0.04	0.74	0.04	***
n-6	12.83	0.90	15.88	0.47	**
C20:5 n-3	0.36	0.01	0.10	0.00	***
C22:5 n-3	1.76	0.13	0.83	0.04	***
C22:6 n-3	0.12	0.01	0.06	0.01	**
n-3	2.70	0.16	1.29	0.05	***
C18:2 9Z-11E	0.32	0.02	0.18	0.01	***
PUFA	15.85	1.03	17.36	0.52	ns
Nutritional important values					
n-6/n-3	4.75	0.14	12.36	0.23	***
PUFA/SFA	0.40	0.03	0.38	0.01	ns

P value of supplier effect. ***: P<0.001, **: P< 0.01, *: P<0.05, ns: P>0.05.

Table 2. Sensory analysis (hedonic test) of cooked beef meat (*longissimus dorsi* muscle) from different suppliers during refrigerated storage.

	0 day		**5th day**		**8th day**	
Attribute	**Supplier 1**	**Supplier 2**	**Supplier 1**	**Supplier 2**	**Supplier 1**	**Supplier 2**
Color	5.52 c*	5.92 bc	5.78 bc	6.26 ab	6.32 ab	6.62 a
Aroma	6.26 abc	5.98 c	6.6 a	6.06 bc	6.5 ab	6.16 bc
Taste	6.38 ab	5.86 bcd	6.28 abc	5.62 d	6.54 a	5.8 cd
Texture	6.2 b	4.68 c	6.4 ab	4.98 c	7.1 a	4.9 c
Acceptability	6.22 a	5.38 b	6.3 a	5.44 b	6.7 a	5.42 b

* Different letters in the same row means significant differences at p<0.05.

Table 3 . Quantification of volatile compounds by SIFT-MS (ppbv) in raw beef from different suppliers during refrigerated storage (values represents the mean of the three animals analyzed in each supplier S1 and S2).

	Day 0		day 2		day 5		day 8		day 12					
Compound	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**SEM[e]**	**Ps[f]**	**Pa**	**Psxa**
aldehydes														
acetaldehyde	12.3 c	16.2 c	12.4 c	26.6 c	19.0 c	40.6 c	51.3 bc	101.2 a	93.7 ab	126.9 a	8.8	***	***	ns
Propanal	1.7 c	2.0 c	1.9 c	1.9 c	1.3 c	4.8 c	3.1 c	14.5 b	6.4 c	86.9 a	1.4	***	***	***
Butanal	1.7 c	1.6 c	1.6 c	2.0 bc	3.3 abc	3.5 abc	4.8 ab	5.6 a	4.9 a	5.9 a	0.6	ns	***	ns
2-butenal	4.9 ab	5.6 ab	4.3 b	5.9 ab	5.0 ab	6.1 ab	5.5 ab	7.1 ab	6.9 ab	8.0 a	0.7	**	*	ns
Pentanal	2.1 b	1.5 b	1.4 b	1.5 bc	2.7 b	4.2 b	3.5 b	7.2 b	6.8 b	22.7 a	1.4	***	***	***
2-pentenal	9.0 b	9.9 b	8.5 b	10.6 b	11.6 b	12.4 b	11.1 b	24.5 a	44.3 a	40.0 a	4.4	ns	***	ns
Hexanal	6.6 c	6.0 c	6.7 c	8.2 c	17.1 c	31.8 c	17.8 c	118.2 bc	174.8 b	429.5 a	28.5	***	***	***
2-hexenal	2.2 cd	4.9 abcd	2.1 d	6.1 abc	2.5 cd	6.5 ab	1.9 d	5.6 abcd	2.8 cbd	7.0 a	0.8	***	ns	ns
Heptanal	2.3 b	2.9 b	2.0 b	2.8 b	1.9 b	4.0 ab	2.5 b	4.5 ab	2.5 b	5.9 a	0.5	***	*	ns
2-heptenal	3.9 c	4.5 c	4.1 c	6.0 c	3.8 c	10.1 bc	5.5 c	16.2 b	9.7 bc	30.1 a	1.8	***	***	***
2-octenal	4.2 b	4.2 b	4.6 ab	5.0 ab	4.9 ab	4.5 ab	4.4 b	6.4 ab	5.6 ab	7.0 a	0.5	*	**	ns
Nonanal	1.9 c	2.1 c	2.5 c	2.9 c	3.0 c	3.9 bc	3.0 bc	5.7 b	4.2 bc	10.5 a	0.2	***	***	***
2-nonenal	5.1 b	4.3 b	4.5 b	5.7 b	5.7 b	5.3 b	5.4 b	6.4 ab	7.1 ab	9.1 a	0.6	ns	***	ns
Decanal	1.5 b	1.8 b	2.4 b	2.7 b	2.3 b	2.3 b	2.0 b	3.6 b	1.8 b	9.4 a	1.1	**	*	**
Esters														
Ethyl acetate	44.7 a	15.0 a	43.8 a	27.7 a	49.0 a	23.3 a	29.9 a	21.0 a	35.3 a	39.2 a	10.3	*	ns	ns
Acids														
Acetic acid	12.9 d	18.1 bcd	13.7 d	17.2 cd	16.9 cd	22.8 bcd	17.2 cd	28.1 abc	30.8 ab	39.1 a	2.7	***	***	ns
Butyric acid	3.4 d	5.2 cd	3.1 d	5.1 cd	4.7 cd	8.3 cd	2.6 d	24.4 ab	16.7 bc	32.0 a	2.2	***	***	***
sulphur compounds														
Dimethyl disulphide	3.7 c	4.7 c	3.1 c	5.0 bc	5.2 bc	11.7 bc	5.4 bc	35.2 abc	52.6 ab	78.5 a	9.5	*	***	ns
Hydrogen sulphide	43.7 a	20.8 ab	42.2 ab	31.9 ab	42.8 ab	30.2 b	27.2 ab	17.7 ab	25.0 ab	22.8 ab	5.2	**	ns	*
Methanethiol	1.9	2.4	2.4	1.9	1.8	2.2	1.9	2.3	2.6	3.1	0.4	ns	ns	ns
Dimethyl sulphide	9.6 d	20.0 d	7.0 d	22.2 cd	12.2 d	30.3 cd	8.7 d	51.8 bc	79.2 b	145.5 a	6.1	***	***	***
Carbon disulphide	30.6 bc	37.3 bc	15.9 c	47.8 bc	21.6 bc	62.4 bc	26.3 bc	79.1 b	43.8 bc	233.8 a	12.6	***	***	***
Ketones														
Acetone	124.9 c	631.2 b	134.6 c	767.2 b	151.0 c	813.4 b	200.5 c	737.1 b	234.8 c	1079.9 a	44.9	***	***	*
2-heptanone	2.9 b	2.1 b	2.1 b	4.1 b	2.6 b	4.4 b	4.2 b	5.8 b	3.5 b	10.3 a	0.7	***	***	***

Alcohols															
Methanol	86.9 [b]	461.3 [a]	134.5 [b]	488.6 [a]	88.6 [b]	484.3 [a]	147.2 [b]	484.2 [a]	185.6 [b]	558.4 [a]	49.4	***	ns	ns
Etanol	279.0	369.7	315.1	362.7	355.8	334.9	313.9	312.5	318.7	367.8	36.4	ns	ns	ns
1-propanol	17.7	234.8	19.6	307.8	21.4	257.6	21.1	339.4	43.7	292.1	71.2	***	ns	ns

[a.-d]: Means with different letters indicate significant differences among storage times. [e] SEM: standard error of the mean, [f] P_s: P value of supplier effect; P_a: P value of refrigerated storage effect; P_{sxa}: P value of interaction between supplier and storage effects. ***: P<0.001, **: P< 0.01, *: P<0.05, ns: P>0.05.

Table 4 .Quantification of volatile compounds by SPME-GC-MS (Abundance units; AU x 10^{-6}) in raw beef from different suppliers during refrigerated storage (values represents the mean of the three animals analyzed in each supplier S1 and S2).

	day 0		day 2		day 5		day 8		day 12					
Compound	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**SEM[e]**	**P_s[f]**	**P_a**	**P_{pxs}**
aldehydes														
Acetaldehyde (45)[g]	2.1 bcd	0.3 d	3.3 bcd	0.8 cd	0.5 d	1.7 cd	5.7 ab	4.5 abc	7.9 a	2.9 bcd	0.8	**	***	*
Propanal (58)	2.0 b	1.6 b	1.6 b	0.9 b	1.4 b	3.5 b	2.2 b	20.1 b	2.4 b	231.0 a	42.4	ns	*	*
Butanal (44)	1.4 b	1.0 b	1.0 b	2.6 b	0.9 b	1.3 b	1.2 b	7.9 b	1.1 b	33.1 a	3.6	**	**	**
Pentanal (44)	7.7 b	10.4 b	5.8 b	8.0 b	3.8 b	22.1 b	7.8 b	161.3 b	19.8 b	640.5 a	66.5	**	**	**
Hexanal 41)	63.3 c	57.1 c	55.9 c	113.4 bc	50.7 c	1565.2 bc	90.4 c	3692.3 b	1008.4 bc	28037.4 a	654.5	***	***	***
2-hexenal (41)	0.9 b	1.1 b	0.7 b	6.6 b	0.4 b	2.1 b	0.8 b	3.9 b	6.2 b	24.5 a	1.4	***	***	***
Heptanal (70)	2.0 b	3.1 b	1.5 b	2.5 b	1.7 b	6.7 b	4.3 b	74.6 b	10.7 b	570.1 a	64.3	**	**	**
2-heptenal (56)		0.4 b		7.4 b	0.4 b	4.2 b	0.3 b	5.5 b	1.9 b	36.9 a	2.7	**	***	***
2-octenal (41)	0.6 b	4.2 b	0.6 b	8.0 b	0.2 b	6.2 b	0.5 b	5.9 b	4.2 b	59.7 a	8.1	**	**	*
Nonanal (57)	4.7 b	4.4 b	5.8 b	17.3 b	6.8 b	61.6 b	14.6 b	173.6 b	48.3 b	733.6 a	53.8	***	***	***
2-nonenal (41)		0.6 b		2.1 b	0.2 b	1.6 b	0.3 b	3.4 b	0.6 b	15.6 a	1.6	**	**	**
Decanal (43)	0.9 b	0.7 b	0.7 b	1.7 ab	0.8 b	2.1 ab	0.8 b	2.0 ab	1.3 b	3.2 a	0.3	***	**	ns
Acids														
acetic acid (60)	99.5 b	62.1 b	84.4 b	86.6 b	106.5 b	80.8 b	290.8 ab	278.1 ab	648.4 a	518.5 ab	79.9	ns	***	ns
butyric acid (60)	2.8	94.2	55.6	76.7	95.8	86.4	47.9	65.4	55.7	51.0	16.2	ns	ns	ns
sulphur compounds														
Methanethiol (47)	0.3 bc	0.4 bc	0.4 bc	0.4 bc	0.2 c	1.0 ab	0.3 c	1.3 a	0.3 c	1.4 a	0.2	***	**	**
dimethyl sulphide (62)	32.1 abcd	49.6 abc	59.6 ab	66.7 a	28.8 abcd	34.9 abcd	32.4 abcd	18.7 cd	23.3 bcd	8.4 d	6.9	ns	***	ns
carbon disulphide (76)	228.5 ab	328.4 a	205.3 ab	230.3 ab	250.9 ab	185.1 ab	297.9 a	125.2 b	243.0 ab	116.9 b	26.7	**	ns	**
Ketones														
Acetone (58)	186.0 c	1290.7 a	200.9 c	1119.0 a	208.7 c	1104.6 a	231.8 c	1259.7 a	285.4 c	694.6 b	64.2	***	*	**
2-heptanone (43)	22.5 d	104.1 cd	29.7 cd	149.6 cd	55.7 cd	534.4 b	151.2 cd	617.6 b	344.3 bc	918.4 a	55.8	***	***	**
Alcohols														
Etanol (31)	12.0 ab	9.3 b	7.2 b	7.4 b	6.2 b	9.1 b	36.3 a	19.8 ab	14.2 ab	22.6 ab	4.7	ns	**	ns
Propanol (31)	3.1 c	3.7 bc	2.1 c	7.1 abc	3.4 c	6.3 abc	4.6 bc	9.9 ab	7.2 abc	12.0 a	1.2	**	**	ns

[a.-d]: Means with different letters indicate significant differences among storage times. [e] SEM: standard error of the mean, [f] P_s: P value of supplier effect; P_a: P value of refrigerated storage effect; P_{sxa}: P value of interaction between supplier and storage effects. ***: P<0.001, **: P< 0.01, *: P<0.05, ns: P>0.05. [g] Number in brackets represents the ion (m/z) used for quantification.

Figure 1

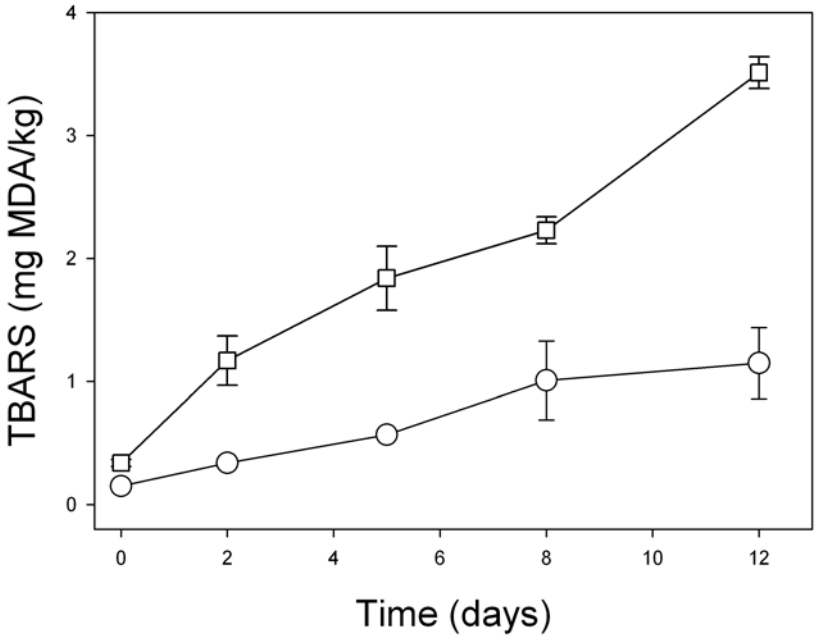

Figure 2