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The good, the bad and the aged: Predicting sensory quality of anhydrous milk fat by PTR/SRI-Tof-MS analysis and data mining

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ABSTRACT

Due to its versatility, anhydrous milk fat (AMF) has become more popular as a food industry ingredient, but its quality control remains a critical challenge. A direct injection mass spectrometry technique was applied to predict sensory quality of AMF. Volatilome analysis through proton transfer reaction mass spectrometry (PTR-MS) was used to classify 39 industrial samples of AMF according to industrial sensory evaluation and to accelerated ageing. A selective reagent ion system was used to evaluate the suitability of PTR-MS alternative ionisation modes for quality control. Supervised multivariate data analysis successfully classified samples and showed that samples exposed to accelerated shelf life at 50 °C presented higher intensities of most volatiles, especially for the ones derived from oxidation like aldehydes and ketones, while samples with an acceptable quality level had lower emissions of volatiles. PTR-MS technique is ideal to support agroindustry sensory quality programs requiring rapid on-line analytical information.

1. Introduction

The global market importance of anhydrous milk fat (AMF) as a food ingredient is continuously increasing due to its high stability and longer shelf life compared with butter, mainly due to its low moisture content (0.2%). For its easy use in liquid form (above 36 °C), the efficient mixing with various other dairy products and its unique sensory properties, AMF is widely used in confectionary, chocolate and ice cream manufacturing industries (Bylung, 1995; Wang et al., 2019).

AMF may differ in quality, functionality and economic value due to (i) milk quality (e.g., seasonality and origin), (ii) manufacturing processes and (iii) duration and conditions of storage. Different instrumental techniques have been developed to characterise physical and chemical properties of butter and AMF such as iodine value, saponification number, moisture content, cis–trans fatty acids ratio, peroxide value, anisidine value, acid value and thiobarbituric acid number (Ismail, van de Voort, Emo, & Sedman, 1993; Koczoñ, Gruczyñska, & Kowalski, 2008). Among them, peroxide and acid values are commonly used to describe AMF quality because they provide an indication of primary oxidation and lipolysis respectively, which are the two most significant deteriorative reactions occurring during storage (FAO, 1984; Koczoñ et al., 2008). These analytical methods, despite being simple and relatively inexpensive, are invasive and require potentially hazardous solvents (Koczoñ et al., 2008). Moreover, they mainly give an indication of product oxidation and therefore about storage and manufacturing conditions. Little information is provided about secondary metabolites such as alcohols, sulphur compounds and aldehydes that could strongly affect AMF sensory characteristics and therefore consumer acceptance of the final products (Murray & Baxter, 2003). For this reason, developments of flexible, fast, and reliable methodologies to monitor the quality focused on flavour and aroma are needed (Munoz, 2002; Reece, 1979). Due to their key role in food perceived quality, volatile organic compounds (VOCs) are of primary interest in the context of monitoring and predicting

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product quality in relation to sensory perception (Biasioli, Yeretzian, Gasperi, & Mark, 2011).

Proton transfer reaction-mass spectrometry (PTR-MS) is a direct injection mass spectrometry method that enables volatile analysis with high sensitivity and in real time (Blake, Monks, & Ellis, 2009; Lindinger, Hansel, & Jordan, 1998). This technique was applied in the past by Van Ruth et al. (2008) for classifying butter and butter oil samples subjected to heat and off-flavour treatment. The same group also proposed the approach for discriminating the geographical origin of European butters (Macatelli et al., 2009). Recently, our group used a similar instrument equipped with a time-of-flight (TOF) mass analyser (Blake, Whyte, Hughes, Ellis, & Monks, 2004; Jordan et al., 2009) to analyse the volatilome of semi-finished dairy ingredients (Makhoul et al., 2016) and to detect differences in VOC release from AMF during storage in different packaging by following changes during the oxidation processes caused by storage conditions at 50 °C (Pedrotti et al., 2018). In this study, we introduce two novel factors: the sensory aspect, where VOC emissions were used to predict quality classes of AMF based on industrial sensory evaluation (quality control) and the usage of a selective reagent ionisation system (SRI). The SRI system allows for the use of different precursor ions (H_3O^+, NO^+, O_2^+, Kr^+ and Xe^+) for ionisation and therefore extends the number of compounds that may be detected, *e.g.* short chain alkanes, and improves the specificity, *e.g.* separating isobaric compounds as ketones and aldehydes (Jordan et al., 2009; Karl et al., 2012; Lindinger et al., 1998). The SRI-ToF-MS system has been applied to a variety of purposes such as identification of monoterpenes (Materić et al., 2017), and identification of new drugs (Lanza et al., 2015) and, in the food area, the technique was successfully applied to improve the discrimination of coffee from different origins (Yener et al., 2015).

In this research, we study a fingerprinting approach based on untargeted PTR-ToF-MS volatilomics to predict different sensory classes of AMF by rapid and non-invasive headspace analysis coupled with data mining methods. Moreover, this study aims to explore if additional information provided by the SRI system can improve performances of predictive models.

2. Material and methods

2.1. AMF samples

Sampling consisted of 25 AMF samples produced by five major European manufactures over two years of production (2015 and 2016). All batches were produced from pasteurised cream with 35%–40% fat content. Anhydrous milk fat was produced by applying the standard method described elsewhere (Bylung, 1995). Samples were divided in two categories based on sensory evaluation: 16 "YES" samples that were classified as good and 9 "NO" samples that were classified as not complying (see the next section for details). Aliquots of some of the "YES" samples were subjected to storage conditions at 50 °C in the dark for 5 days in a thermostatic stove to simulate aging and to create defective samples through thermal oxidation. These samples were called "AGED" (14 samples). Samples were then packed in plastic bags for shipment and transported on ice packs to the analytical laboratory at Fondazione Edmund Mach (San Michele all'Adige, Italy). Upon receipt, products were removed from shipping containers, examined for damage, and assigned to frozen storage (−20 ± 1 °C) until analysis.

2.2. Sensory analysis

Sensory evaluation was carried out by 7–12 trained judges (26.8% females, age range 30–55 years old) according to the industrial partner internal evaluation protocol based on a 'difference from control' rating (Lawless & Heymann, 2010). Each AMF sample was rated by comparison with a standard. About 200 g of each sample were melted and kept at 50 °C in a thermostatic stove and a spoon (around 15 g) of melted AMF was served to each participant. Panellists were instructed to evaluate the odour intensity of the samples first. Then, judges assessed flavour intensity by taking a sip of the sample. Between samples, judges rinsed their mouth with water to remove all fat residues. The difference from the standard reference sample was evaluated on a linear continuous scale from 0 to 5. For each sample average scores from the panel were obtained. Based on this average and using a cut-off value of 1.5 established by industrial internal standards, AMF scoring higher than 1.5 were classified as "NO" while AMF scoring lower were classified as "YES". In Table 1 a list of the samples is given.

2.3. PTR/SRI-ToF -MS analysis

According to industry practice, each sample was melted in a thermal bath (50 °C) and fifteen 2.5-mL aliquots of AMF were transferred into sampling vials, which were previously conditioned for 1 day at 65 °C. Vials were then closed, labelled and kept at 4 °C till analysis. Empty vials were used as blanks and five replicates were measured for each reagent ion. All vials were incubated for

Table 1
Anhydrous milkfat samples used, their sensory score, production date, supplier and classification based on the sensory test.

Sample number	Classification	Supplier	Production date	Sensory score
12	NO	A	20-03-15	3
18	NO	A	26-01-15	1.7
20	NO	B	03-06-15	3.3
22	NO	C	03-06-15	1.5
A	NO	ND	ND	1.6
B	NO	ND	ND	1.6
C	NO	ND	ND	1.7
D	NO	ND	ND	1.6
1	YES	ND	ND	1.0
2	NO	ND	ND	1.5
3	YES	ND	ND	1.1
46	YES	A	31-08-16	0.9
47	YES	C	30-10-16	0.6
48	YES	D	07-11-16	0.9
49	YES	A	07-11-16	0.8
50	YES	C	20-12-16	0.6
51	YES	A	09-01-17	0.9
45	YES	A	14-11-16	1.0
38	YES	C	01-08-16	0.7
39	YES	A	30-08-16	1.1
40	YES	A	06-07-16	0.9
41	YES	C	11-07-16	0.7
43	YES	E	27-07-16	1.0
52	YES	D	30-07-16	1.3
53	YES	A	27-02-17	0.8
46-i	AGED	A	31-08-16	1.5
47-i	AGED	C	30-10-16	1.5
48-i	AGED	D	07-11-16	1.5
49-i	AGED	A	07-11-16	1.6
50-i	AGED	C	20-12-16	1.3
51-i	AGED	A	09-01-17	1.7
45-i	AGED	A	14-11-16	2.2
38-i	AGED	C	01-08-16	1.5
39-i	AGED	A	30-08-16	1.7
40-i	AGED	A	06-07-16	2.5
41-i	AGED	C	11-07-16	1.3
43-i	AGED	E	27-07-16	1.4
52-i	AGED	D	30-07-16	1.5
53-i	AGED	A	27-02-17	1.6

[a] Samples which in the sensory test scored higher than the quality control cutoff score of 1.5 were classified as "NO", while the one which scored below 1.5 as "YES". The "AGED" samples were obtained from the "YES" samples after 5 days in a thermostatic stove at 50 °C to simulate ageing.

equilibration at 50 °C for at least 30 min before PTR-MS analysis. Each sample was measured for 60 s with an acquisition rate of one spectrum per second and a flow rate of 35 sccm. To avoid memory effects, measurement order was randomised and, after each measurement, a waiting time of 3 min was set.

All measurements were performed using a multipurpose GC sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) connected to the inlet of the PTR-ToF-MS as previously described (Yener et al., 2014). The inlet line consisted of a PEEK capillary tube (inner diameter, 0.40 mm), heated at 110 °C.

A PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) in its standard configuration (V mode) was used. The instrument was equipped with a switchable reagent ion system (SRI) that allowed the operation in H_3O^+, NO^+ or O_2^+ modes as described elsewhere (Cappellin et al., 2014; Sánchez del Pulgar et al., 2013). The current applied for the discharge in the ion source was 3.5 mA in the case of H_3O^+ while it was set at 5.0 mA in the case of NO^+ and O_2^+ modes. Drift conditions were the same for all three ionisation conditions: drift voltage = 557 V, drift temperature = 110 °C, drift pressure = 2.3 mbar (except for the NO^+ that was 2.8 mbar) resulting in an E/N value of 141 Td (136 Td for NO^+ mode). Mass resolution (m Δm^{-1}) was at least 3800, and data were collected for the mass range *m/z* 20 to 300.

2.4. Data processing

Data processing of ToF spectra included dead time correction, internal calibration of mass spectral data and peak extraction (Cappellin et al., 2010, 2011) to reach a mass accuracy sufficient for determining sum formula of volatile compounds. In this paper, the experimental *m/z* values are reported up to the third decimal place. The *m/z* axis internal calibration was performed using *m/z* 21.0221, 29.9974, and 203.9430 corresponding to protonated water, NO^+, and one of the fragments of 1,3-diodobenzene, which was continuously injected as a reference compound through the PerMaSCal device (Ionicon). Peak intensities from the absolute mass spectra were converted to concentrations in ppbV (parts per billion by volume) according to the procedure reported in Lindinger et al. (1998), assuming a constant reaction rate coefficient ($k = 2 \times 10^{-9}$ cm^3 s^{-1}) and by averaging mass spectral signals over 40 spectra. This approximation introduces a systematic error for the absolute concentration of each compound that is in most cases below 30% (Biasioli et al., 2006; Cappellin et al., 2012).

The data extraction of PTR\SRI-ToF-MS spectra provided 519 mass peaks for H_3O^+, 445 for O_2^+ and 461 for NO^+. The matrix consisting of concentration estimation for each sample and each peak ("raw data"), was further processed to extract relevant information and reduce the noise signals associated with PTR-MS measurements. A preliminary reduction of the data set was obtained by excluding (i) all peaks with a concentration significantly lower than blanks (t.test with $p < 0.01$ after Bonferroni correction for multiple tests), (ii) ^{13}C isotopologues and (iii) signals related to interfering ions at *m/z* 29.997 (NO^+), 32.998 (O_2^+ isotope), 37.032, 55.039, 73.050 (all water clusters) and 51.043 (methanol cluster). This procedure allowed selection of 120 mass peaks for H_3O^+, 73 for O_2^+ and 84 for NO^+. If not stated differently, statistical analyses were performed on these "reduced data" sets.

2.5. Data analysis

2.5.1. PCA and univariate data analysis

Preliminary principal components analysis (PCA) was performed after logarithmic transformation and scaling (mean centring and unit variance). After checking assumptions, one-way ANOVA with Tukey honest significant difference (HSD) and Bonferroni corrections were performed to find the mass peaks that were significantly different among the AMF sensory classes. Of these, the ones with $m/z > 33$ and with a concentration threshold equal or above to 0.5 ppbV in at least two classes, were selected to build summary tables. Tentative peak identification was performed using an in-house library developed by the authors and available literature (Beauchamp, Zardin, Silcock, & Bremer, 2014; Krause, Miracle, Sanders, Dean, & Drake, 2008; Makhoul et al., 2016; Pedrotti et al., 2018; Peterson & Reineccius, 2003a; Van Ruth et al., 2008; Widder, Sen, & Grosch, 1991).

A spider plot for the log transformed data obtained with H_3O^+ as reagent ion was built based on the mass peaks selected by univariate data analysis and with a concentration higher than 1 ppbV. Total ion count (TIC) obtained by summing the selected mass peaks for each sample was included the in the graph as well.

2.5.2. PLS-DA classification

Finally, a supervised classification method, namely partial least square regression-discriminant analysis (PLS-DA), was carried out for samples classification. The analysis was applied to the datasets obtained with the three different reagent ions on both "raw data" and "reduced data" resulting from the data elaboration process. Each dataset was firstly divided into training and test sets with a proportion of 80% and 20% of the samples, respectively. Then, using the training set, the model was trained via applying a three-fold cross-validation procedure iterated 100 times to extract classification error rates to adjust the discrimination method and chose the models' number of components. The trained models were then used to predict the samples sensory classes of the corresponding test sets. The whole procedure was iterated 1000 times for all datasets. Results were evaluated using mean classification errors, mean balanced error rates (BER) and confusion matrices of each model. The BER is used when the samples classes are unbalanced since it calculates the average proportion of wrongly classified samples in each class, weighted by the number of samples in each class (Rohart, Gautier, Singh, & Lê Cao, 2017). In addition to the individual analysis of the three precursor ions, the same data were merged into a multi-precursor dataset, following a data fusion strategy (Borràs et al., 2015). Particularly, a "low-level" fusion was applied: data from the 3 different ionisation modes were concatenated sample-wise into a single matrix. This was done separately for data sets of "raw data" and for "reduced data". As for the PLS-DA performed previously, the matrixes were pre-processed through auto-scaling. The prediction models were run on all the three classes and as well by only considering the "YES" *vs* "NO" case.

All analyses and graphs were performed with core functions of R programming language and its external packages (R Core Team, 2016) (ChemometricsWithR, mixOmics, multcomp, vegan, matrixStats, ggplot2) and Excel (version 14.0.7224.5000).

3. Results and discussion

3.1. Samples classification: sensory and aging effect

The mass peaks in the "reduced data" sets were chosen as variables, and two principal components (PC) were produced. The score plots obtained for PC1 and PC2 corresponding to each ionisation mode (H_3O^+, NO^+ and O_2^+) are shown in Fig. 1.

As presented in Fig. 1, for all PCAs the first two PCs explain more than 50% of the total variability and the three different sample classes (YES, NO and AGED) are represented with different colours. In the case of H_3O^+, the PCA indicates the presences of some clusters: the PC1 separates the AGED samples from the original ones (especially when looking at Fig. 1A) while the PC2 separates the "YES" class from the "NO" class. This latter distinction is even

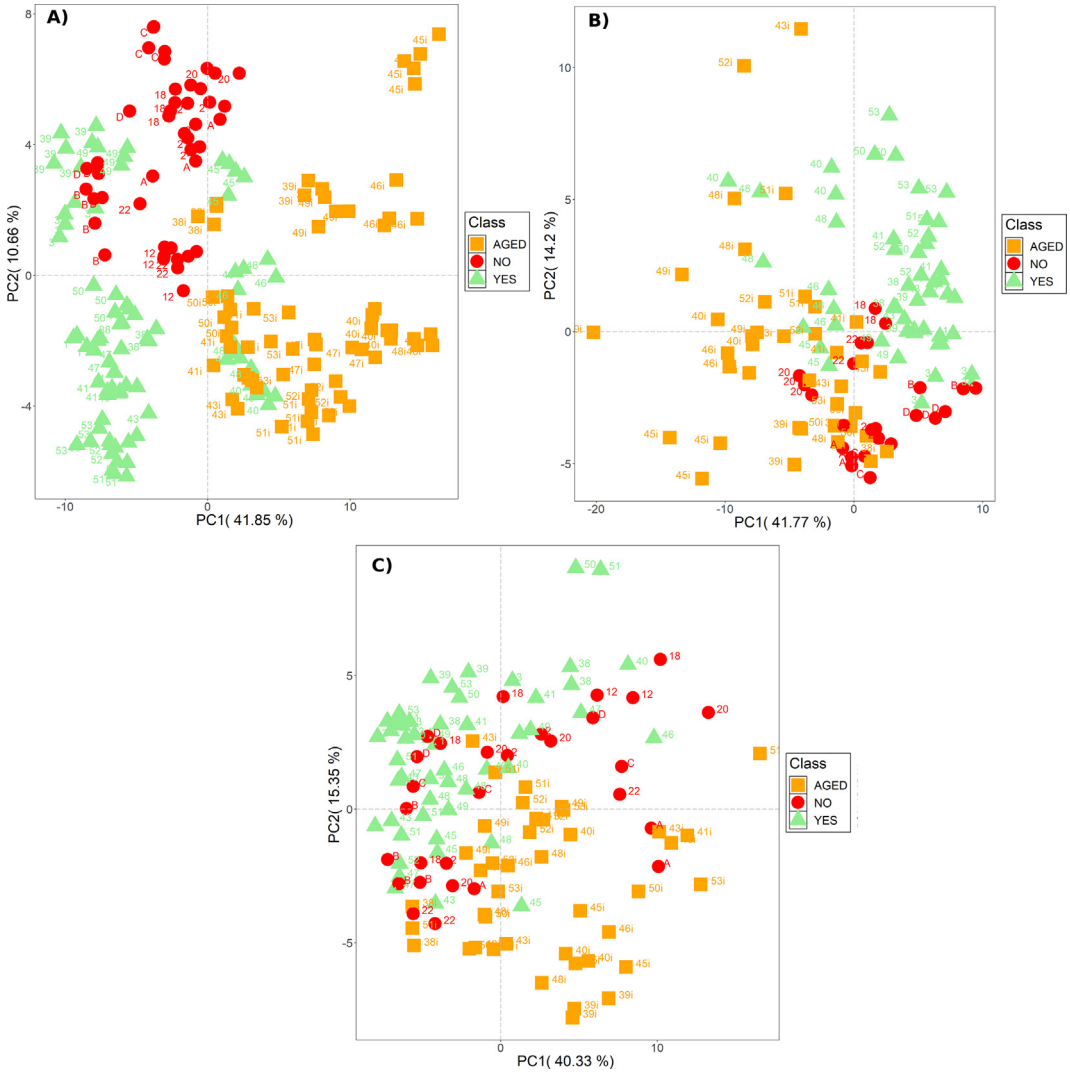

Fig. 1. Score plots of principal component analysis (PCA) for the 3 ionisation modes: (A) H_3O^+ mode, (B) NO^+ mode, (C) O_2^+ mode. The first two principal components are shown. The different colours and the different shapes indicate the different AMF classes (■, AGED; ●, NO; ▲, YES). For each sample the five replicates are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

better when the NO^+ mode was used (Fig. 1B). It is also possible to notice some internal structure in the "YES" class for the H_3O^+ mode that is associated to the different suppliers but it is not considered here.

One-way ANOVA followed by Tukey HSD (with Bonferroni correction) was conducted to further evaluate differences in VOCs among the three sample classes for all the reagent ions. Mass peaks that were significantly different for at least one class ($p < 0.01$) were reported in Table 2. Of the 49 mass peaks listed, 36 were tentatively identified on the basis of their sum formula, isotopic and fragmentation pattern, our internal database and literature data. Results for O_2^+ and NO^+ are reported in supplementary materials (see Supplementary material Tables S1 and S4).

From Table 2 can be seen that the intensity of most peaks is higher for "AGED" samples than for the other classes (59%). The same results were found for the measurements using O_2^+ and NO^+ as precursor ions, indicating the importance of oxidation phenomena during heat treatment in increasing the concentration of different VOCs (Supplementary material Tables S1 and S4).

The most abundant mass peaks found in the headspace of the "AGED" AMFs, measured with H_3O^+ ion, were *m/z* 45.033, 73.065 and 87.081, tentatively identified as acetaldehyde, 2-butanone/butanal and 2-pentanone/diacetyl/2-/3-methylbutanal, respectively. Such compounds were all described as key butter aroma compounds (Maarse, Visscher, Willemsens, & Boelens, 1989; Mallia, Escher, & Schlichtherle-Cerny, 2008; Schieberle, Gassenmeier, Guth, Sen, & Grosch, 1993) and were previously found in a similar experiment (Pedrotti et al., 2018). In particular, 2-butanone and acetone in milk have been reported to originate from cows' feed (Marsili, 2011) while acetaldehyde, diacetyl, 2- and 3-methylbutanal were found as important contributors of the aroma in sweet cream butter (Peterson & Reineccius, 2003b) and some of them were reported to increase during storage at 4 °C (Lozano, Miracle, Krause, Drake, & Cadwallader, 2007). The mass peak *m/z* 73.065, was the most concentrated compound as well in the "YES" class. This class had a significantly lower concentration of *m/z* 73.065 than the "AGED" class but was significantly higher than the "NO" class. In this case, the SRI analysis can add relevant information where, using NO^+ as reagent ion, is possible to achieve a separation of aldehydes and ketones since different reactions occurs (aldehydes undergo mostly a charge−transfer reaction with NO^+ while ketones are subjected to ion−molecule association reactions).

Table 2
Tentatively identified mass peaks from the headspace analysis of anhydrous milk fat.[a]

Mass	Theoretical mass	Chemical formula	AGED	NO	YES	Tentative identification
33.033	33.0339	CH_4OH^+	33.5 ± 26.9[a]	217 ± 261.3[c]	71 ± 153.9[b]	Methanol
41.038	41.0391	$C_3H_5^+$	29.8 ± 25.9 [ab]	23.7 ± 119.1[b]	13.5 ± 12.5[a]	Alkyl alcohol fragment
42.034	42.0344	$C_2H_3NH^+$	1.2±1[a]	5.8 ± 14[b]	2.1 ± 1.9[a]	Acetonitrile
43.018	43.0184	$C_2H_3O^+$	61 ± 27.8[b]	32.7 ± 9.6[a]	24.1 ± 25.3[a]	Acetic acid fragment
43.054	43.0548	$C_3H_7^+$	8.6 ± 2.9[a]	13.4 ± 128.4[b]	7 ± 4.4[a]	Alkyl fragment
45.033	45.0340	$C_2H_4OH^+$	166.6 ± 74.1[b]	71.9 ± 17.9[a]	39.6 ± 37.9[a]	Acetaldehyde
45.992	45.9871	$CHSH^+$	1.22 ± 0.06[b]	1.17 ± 0.04[a]	1.19 ± 0.05[a]	–
46.029	46.0287	CH_3NOH^+	1.3 ± 0.5[b]	1.5 ± 0.3[b]	1.3 ± 0.2a	–
47.013	47.0128	$CH_2O_2H^+$	9.5 ± 5.4[b]	9.4 ± 2.5 [ab]	8.8 ± 1.7[a]	Formaldehyde
47.025	47.0245	$H_2N_2OH^+$	3 ± 0.3[b]	2.8 ± 0.2[a]	3.1 ± 0.3[b]	–
47.049	47.0497	$C_2H_6OH^+$	24.78 ± 41.05 [ab]	57.34 ± 64.42[a]	26.63 ± 74.45[b]	Ethanol
49.011	49.0112	CH_5S^+	2.4 ± 1.5[c]	0.7 ± 0.5[a]	1.4 ± 0.8[b]	Methanethiol
53.039	53.0391	$C_4H_4^+$	1.1 ± 0.4[b]	0.6 ± 0.2[a]	0.5 ± 0.2[a]	–
55.055	55.0548	$C_4H_6H^+$	25.2 ± 8.4[b]	13.7 ± 4.2[a]	10.1 ± 4.8[a]	Butanal fragment
57.034	57.0340	$C_3H_4OH^+$	5.6 ± 8.5[b]	1.4 ± 1.5[a]	1.1 ± 0.8[a]	2-Propenal/Acetol fragment
57.07	57.0699	$C_4H_9^+$	3.7 ± 1.4[b]	3.4 ± 1.5 [ab]	3.1 ± 0.9[a]	Common fragment (alcohol, ester)
58.041	58.0419	$C_3H_5OH^+$	2.1 ± 0.9[b]	0.7 ± 0.3[a]	0.3 ± 0.4[a]	–
60.053	60.0525	C_2 [13]CH_6OH^+	87.7 ± 29.7[b]	15.7 ± 12.5[a]	21.5 ± 21.3[a]	2-Propanone isotope
61.028	61.0290	$C_2H_4O_2H^+$	37.5 ± 38.2[b]	29.7 ± 14.7 [ab]	20.3 ± 35.1[a]	Acetic acid/Acetate
63.027	63.0268	$C_2H_6SH^+$	9.1 ± 3.5[b]	2.4 ± 0.8[a]	9.8 ± 5.7[b]	Ethanethiol
67.055	67.0547	$C_5H_7^+$	0.9±1[b]	0.6 ± 0.3[a]	0.5 ± 0.5[a]	2-Pentanal fragment
69.07	69.0704	$C_5H_8H^+$	14.8 ± 2.71[b]	7.9 ± 8.9[a]	4.8 ± 15.5[a]	Isoprene/3-Hexen-2-ol
71.049	71.0491	$C_4H_6OH^+$	3.2 ± 1.5[b]	1.7 ± 0.6[a]	1.4 ± 0.5[a]	2-Butenal/2,3-Butadien-1-ol/2-Butenal
71.086	71.0855	$C_5H_{10}H^+$	1.3 ± 0.2[b]	0.8 ± 0.3[a]	0.9 ± 0.3[a]	1 (2)-Pentene
73.028	73.0284	$C_3H_4O_2H^+$	1.1 ± 0.4[b]	0.7 ± 0.2[a]	0.7 ± 0.1[a]	Propiolactone
73.065	73.0653	$C_4H_8OH^+$	147.7 ± 26.8[c]	66.9 ± 17.5[a]	97.2 ± 30.3[b]	2-Butanone/Butanal
75.029	75.0263	$C_3H_6SH^+$	1.9 ± 0.3[a]	2 ± 0.2[b]	1.8 ± 0.3[a]	–
75.044	75.0441	$C_3H_6O_2H^+$	3.1 ± 3.1[b]	3.6 ± 3.2[b]	1.7±1[a]	Propanoic Acid
79.054	79.0542	$C_6H_6H^+$	0.8 ± 0.2[b]	0.9 ± 0.2[c]	0.7 ± 0.1[a]	Benzene
82.945	82.9950	$C_4H_3S^+$	0.6 ± 0.2[b]	0.5 ± 0.1[a]	0.6 ± 0.2[b]	–
83.087	83.0860	$C_6H_{11}^+$	3.4 ± 2.8[b]	2.1 ± 1.6[a]	1.9 ± 1.4[a]	Hexanal fragment
85.066	85.0653	$C_5H_8OH^+$	1.1 ± 0.8[b]	0.6 ± 0.2[a]	0.4 ± 0.2[a]	2-Pentenal (E)/1-penten-3-one
87.044	87.0401	$C_4H_6O_2H^+$	4.2 ± 1.9[b]	1.1 ± 0.8[a]	1.2 ± 0.8[a]	2,3-Butanedione/γ-Butyrolactone
87.081	87.0809	$C_5H_{10}OH^+$	143.3 ± 46.9[b]	51.3 ± 27.7[a]	23.7 ± 33.6[a]	2-Pentanone/2/3-methylbutanal
89.061	89.0603	$C4H_8O_2H^+$	9.7 ± 5.1[c]	7.5 ± 2.7[b]	5 ± 2.9[a]	Butanoic acid/Acetoin
90.95			1.7 ± 0.1[b]	1.8 ± 0.1[b]	1.7 ± 0.1[a]	–
93.071	93.0704	$C_7H_8H^+$	1.3 ± 0.9[a]	2.1 ± 2.8[b]	1.2 ± 3.6 [ab]	Toluene (methyl benzene)
96.961	96.9612	$C_2H_2Cl_2H^+$	0.1 ± 0.6[a]	1.1 ± 0.7[b]	0.1 ± 0.3[a]	Dichloroethylene
97.103	97.1012	$C_7H_{13}^+$	5.8±2[b]	2.9 ± 1.2[a]	1.5 ± 1.3[a]	Heptanal fragment
101.061	101.0597	$C_5H_9O_2H^+$	1 ± 0.4[b]	0.8 ± 0.1[a]	0.7 ± 0.1[a]	2,3-Pentanedione
101.097	101.0966	$C_6H_{12}OH^+$	3.3 ± 1.3[b]	1.6 ± 0.6[a]	0.7 ± 0.9[a]	Hexanal
107.087	107.0497	$C_7H_6OH^+$	0.4 ± 0.3[a]	0.8 ± 1.2[b]	0.5 ± 0.3[a]	Benzaldehyde/1,3 Dimethylbenzene
108.957			0.89 ± 0.05[b]	0.86 ± 0.04[a]	0.91 ± 0.04[b]	–
109.103	109.1012	$C_8H_{13}^+$	0.49 ± 0.11 [ab]	0.5 ± 0.22[b]	0.47 ± 0.12[a]	–
115.085	115.0867	$C_5H_{10}N_2OH^+$	0.6 ± 0.2[b]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	–
115.114	115.1117	$C_7H_{14}OH^+$	63.4 ± 22.5[b]	27.9 ± 14.9[a]	12.4 ± 16.2[a]	2-Heptanone
143.147	143.1432	$C_9H_{18}OH^+$	1.5 ± 0.5[b]	0.9 ± 0.3[a]	0.8 ± 0.3[a]	2-Nonanone

[a] The mass peaks that were found significantly different among the classes (indicated by different superscript letters; $p < 0.01$) and having a concentration above 0.5 ppbV in at least two classes were selected.

This separation is shown in the boxplots in Fig. 2 for mass peak *m/z* 73.065: the aldehyde proportion (corresponding to butanal) at *m/z* 71.049 and the ketone (2-butanone) at *m/z* 102.054. A different trend is observed for the two compounds: the ketone in the "YES" class has higher concentrations ($p < 0.01$) than the "NO" class, while for the aldehyde the "YES" class has lower levels although only marginally significant ($p < 0.03$). Different levels of 2-butanone may be due to the different type of feed that was provided to the cows (Marsili, 2011; Villeneuve et al., 2013). These data indicate that the 2-butanone may be recognised by the panellists as a marker of freshness since it has been associated with buttery odour (O'Callaghan et al., 2016). The difference in concentration for the different classes makes the ketone a potential quality marker; the compound needs to be over a certain concentration to meet industrial quality criteria but may be critical when samples reach higher concentrations possibly induced by thermo-oxidation processes.

NO^+ ionisation mode also led to a separation of the mass peak *m/z* 115.113 tentatively identified as a mixture of heptanal and 2-heptanone, a potent odour-active compounds in dairy products (Pedrotti et al., 2018). In this case the aldehyde and the ketone did not have different trends in the AMF classes but 2-heptanone had higher concentrations than the aldehyde. 2-Heptanone has been reported in many different studies on both butter and butter oil aroma (Macatelli et al., 2009; O'Callaghan et al., 2016; Peterson & Reineccius, 2003b) and has been described as having a dairy-like odour (Mallia et al., 2008).

The spider plot in Fig. 3 summarises the profile of significant ($p < 0.05$) VOCs with concentration greater than 1.0 ppbV for the three different classes of AMF samples for the H_3O^+ mode. As already seen in the table, the "AGED" class presented more compounds with higher concentrations due to oxidation induced by the thermal treatment. This is summarised also by the TIC in the spider plot obtained by summing the signal of all these mass peaks: the "AGED" class has the highest levels, followed by the "NO" samples, which also have some mass peaks with the highest levels.

An illustrative example of this trend is *m/z* 101.098 tentatively identified as hexanal, one of the most studied volatile biomarkers

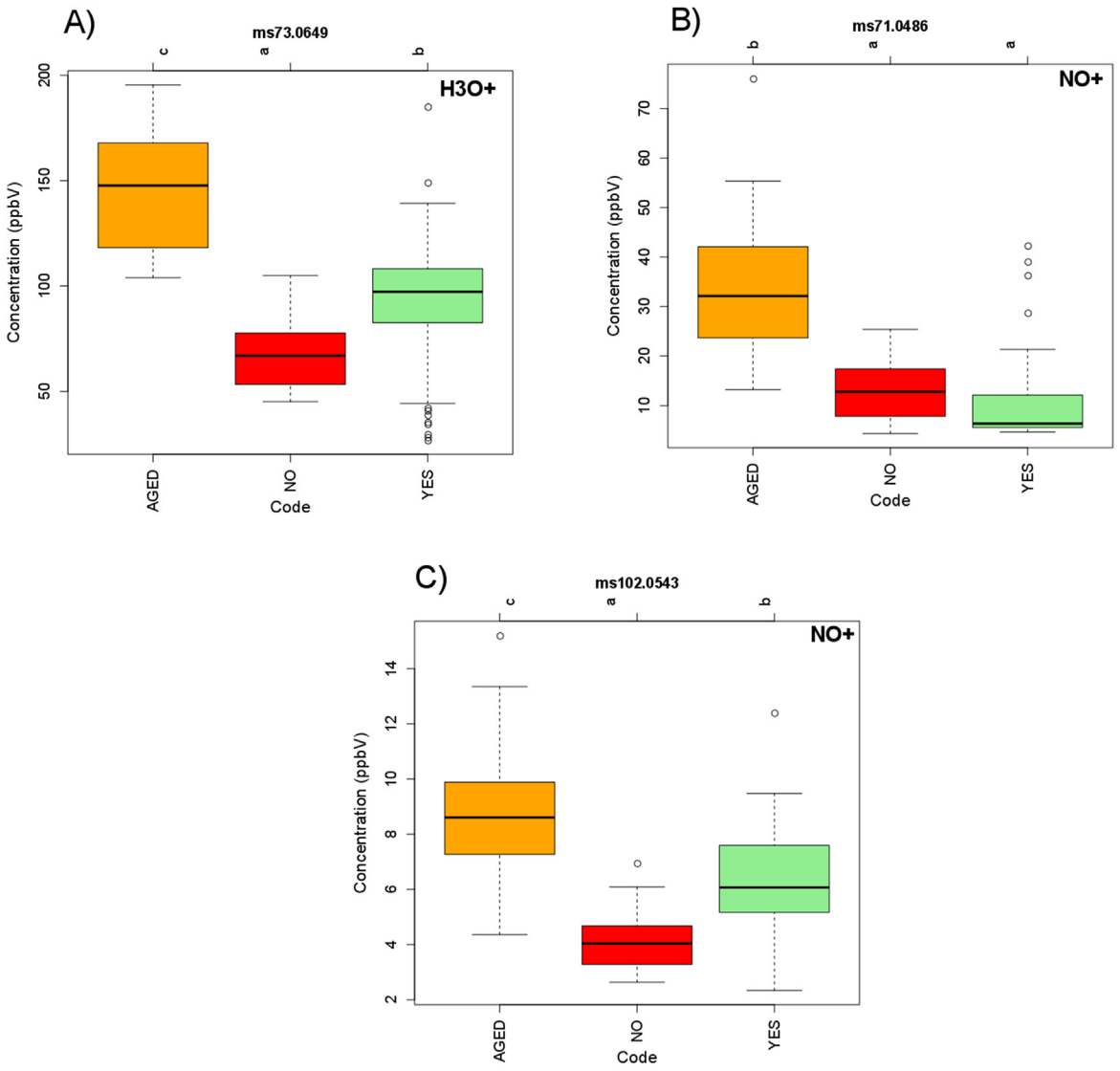

Fig. 2. Boxplots of relevant AMF mass peaks: (A) $m/z = 73.065$, $C_4H_8OH^+$ obtained with H_3O^+ as reagent ion, tentatively identified as a mixture of 2-butanone and butanal; (B) $m/z = 71.049$, $C_4H_7O^+$ tentatively identified as butanal resulting from the hydride ion transfer reaction obtained with NO^+ as reagent ion; (C) $m/z = 102.054$, $C_4H_8ONO^+$ tentatively identified as 2-butanaone resulting from the ion–molecule association obtained with NO^+ as reagent ion.

for dairy product oxidation. Several studies confirmed that this compound, which has been associated with grassy/metallic-like off-flavour in butter and milk, increases during storage (Asaduzzaman, Biasioli, Cosio, & Schampicchio, 2017; Correddu, Nudda, Manca, Pulina, & Dalsgaard, 2015; Garcia-Llatas, Lagarda, Romero, Abellan, & Farre, 2007; Panseri, Soncin, Chiesa, & Biondi, 2011). Hexanal is produced from the auto-oxidation of n-6 poly-unsaturated fatty acids (Contarini, Povolo, Leardi, & Toppino, 1997) and therefore it is not surprising that storage at 50 °C boosted its formation.

In general, the "AGED" class had the highest concentration for most of the mass peaks, followed by the "NO" class which had higher levels than the "YES" class. This is in agreement with the sensory analysis results of the industrial partner where the "gold" standard for a raw material is to have a neutral flavour as much as possible, without any off-notes. Therefore, samples are penalised when they present strong flavours.

The "NO" class presented higher levels for m/z 33.033 that was tentatively associated with methanol. To the authors knowledge no previous GC–MS studies on milk fat or butter reported the presence of this compound in the food matrixes. This may be due to a different number of analytical factors related to the GC–MS methods. For example, some SPME fibres do not have an high affinity for this compound, methanol can be used as a solvent for extractions of VOCs or, due to its psychochemical characteristics (i.e., low molecular weight, high volatility), the compound may elute under the air peak or at the beginning of the chromatogram and then is not been considered in the analysis. On the other hand, PTR-MS has found methanol in both AMF and milk powders (Liu, Koot, Hettinga, de Jong, & van Ruth, 2018; Makhoul et al., 2016; Pedrotti et al., 2018). Methanol formation from pectin has been reported in cow's rumen fermentation from the hydrolysis of methyl esters by rumen microorganisms (Vantcheva, Pradhan, & Hemken, 2010). The methanol may then be transferred to the milk and then to the AMF although at very low concentration. In the same study, it was also suggested that diets with different pectin contents may lead to differences in methanol formation. In our case the "NO" samples may then come from cows which were fed diets containing higher pectin contents. Other mass peaks that have a higher concentration (but still < 5.0 ppbV) in the headspace of the

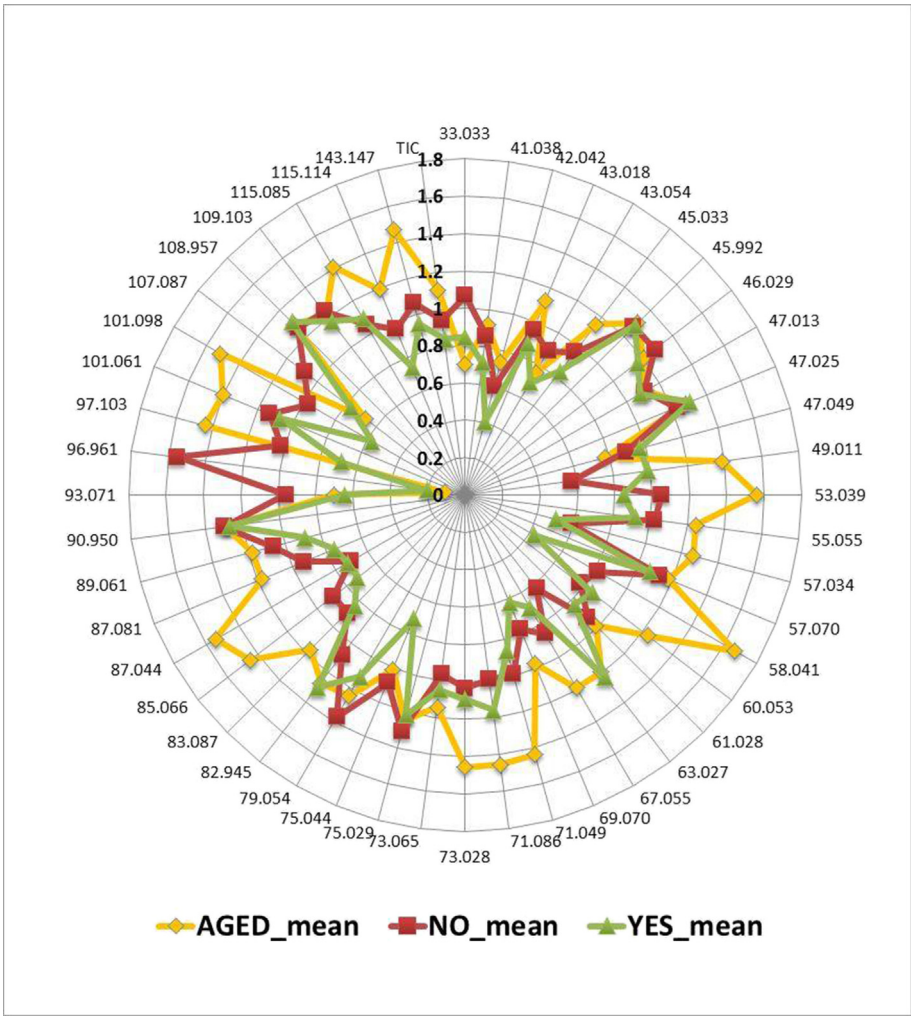

Fig. 3. H_3O^+ mode. Spiderplot of mass peaks selected from 1-way ANOVA and using a threshold value of 0.5 ppbV. Values were centered and log scaled before being plotted (◆, AGED; ■, NO; ▲, YES).

"NO" class are those associated to *m/z* 79.054 and 93.071, tentatively associated to aromatic hydrocarbons like benzene and toluene and possibly fragments of higher aromatic compounds or of other hydrocarbons (i.e., ethylbenzene, n-ethyltoluene, propylbenenze) (Gueneron, Erickson, Vanderschelden, & Jobson, 2015).

Aromatic hydrocarbons are known environmental contaminants in food due to their high volatility and attraction to fats. Benzene and toluene have been reported in different food volatile mixtures (McNeal, Nyman, Diachenko, & Hollifield, 1993) and have been found in traces in butter, heated butter and milk in previous investigations with different analytical techniques (Fabietti, Delise, & Bocca, 2000; Faulkner et al., 2018; Forss, 2009; Lee, Macku, & Shibamoto, 1991; O'Callaghan et al., 2016). As well, Villenueve et al. (2013) found that pasture-fed cows produced milk with a higher level of toluene then cows fed hay and silage. Toluene could be a product of β-carotene degradation (Daun, 2005). The processing of hay and silage forages are known to degrade carotenoids and so the diet type may lead to differences in milk concentrations of carotenoid, leading to differences in toluene levels in the milk (Nozière et al., 2006). In our case, these differences in toluene concentrations have been transferred to the anhydrous milk fat and stress again the preeminent role of cow diet on the quality of the processed product.

Finally, another mass peak that had higher levels in the "NO" class and that can be used as another quality marker in the model definition is the *m/z* 96.961. This mass peak has been associated with a chloride compound ($C_2H_2Cl_2H^+$) based on *m/z* value and its isotopic ratios. The compound may be a fragment of one or more organochlorine pesticides residues like chlorinated derivatives of diphenyl ethane, the groups of hexachlorobenzene, hexachlorocyclohexanes and cyclodienes (Rêgo et al., 2019). Despite these pesticides has been banished for usage in agriculture in most countries worldwide, occurrence in different milk samples have been registered due to water contamination, use of pesticides in the control of ectoparasites directly in the animal and consumption of contaminated pastures and/or rations since the rates of reduction in organochlorine levels in the terrestrial environment are slow (Bajwa & Sandhu, 2014; Rêgo et al., 2019).

The mentioned mass peaks may then be of primary importance for industrial quality control: they can help in discriminating samples of lower sensory quality although the link with sensory attribute cannot be direct. In fact, these defects may originate during the different steps of the supply chain or they may be the result of different types of cow feed (Faulkner et al., 2018). Also, they may come from latter stages during the raw material processes at the manufacturer level. Finally, non-optimal storage conditions and different packaging materials (Pedrotti et al., 2018) may also

led to increasing concentrations of these VOCs. Some of the highlighted markers may be directly correlated to the sensory perception and evaluation of the samples (i.e., hexanal, 2/3-methyl butanone) while some others may not be perceivable to the panel. The latter may be associated to the usage of solvents and pesticides or to the non-optimal storage of the samples and, despite of not being associated to any sensory aspects, may add relevant information for a classification model.

3.2. PLS-DA classification

3.2.1. YES versus NO versus AGED

Cross-validation of the models resulted in classification performances between 9 and 22% with the lowest error for the NO^+ mode (see Supplementary material Table S3). Data obtained with H_3O^+ and O_2^+ ionisation modes presented a BER similar to the aggregate data ("reduced data") matrix. Using the "reduced data" provided better classification results for the NO^+ and the O_2^+ ionisation mode, but it was the opposite for the H_3O^+ mode. The confusion matrixes are presented in Table 3 for the "YES" versus "NO" comparison for the 3 ionisation modes and the aggregate data matrix; Table 3 also contains the classification rates averages for each classification model. The data for the comparison also with the "AGED" samples can be found the Supplementary material Table S4.

3.2.2. YES versus NO

While the "AGED" class is relevant for observing how thermo-oxidation phenomena can affect the aroma profile, for industrial quality control purposes the "YES" versus "NO" classes comparison is more relevant. For this reason, all statistical analyses were repeated by considering just this case.

PLS-DA with two classes "YES" and "NO" has a considerably higher classification rate reaching a minimum average BER of 4% for NO^+ mode when all mass peaks were considered (Table S3). Confusion matrixes in Table 3 indicate that for this ionisation mode, when "raw data" were included in the model, just 3% of the samples were misclassified on average. The NO^+ ionisation mode has the best performance also when using "reduced data set". The reason for a better performance of the NO^+ mode may lay in the fact that this ionisation mode provides an enhanced separation of aldehydes and ketones that are known to be critical in determining perceived flavour of food (Diez-Simon, Mumm, & Hall, 2019). It may then be interesting from an industrial point of view to have a closer look at the SRI system for quality control purposes. For the other ionisation modes and the aggregated data matrix, the "NO" class, the most critical class for a quality control program, had a slighter higher chance to be misclassified then the "YES" class. When looking more in details at the misclassified samples, the sample "B" (sensory score 1.6), followed by sample "3", "49" and "18" (sensory score: 1.1, 0.8, 1.7) were the ones that, across all models, had the highest rate of misclassification. Most of these samples have scores near the acceptability threshold of 1.5 suggesting a good reliability of the predictive models built from PTR/SRI-TOF-MS data.

Univariate data analysis confirms that for H_3O^+ mode "NO" samples presented a higher portion of mass peaks with higher concentrations (75%, see Table 2). On the other hand, some markers with a significantly higher concentration in the "YES" class were identified ($p > 0.001$). In particular, when looking at compounds with relevant concentrations (>1 ppbV) two mass peaks were identified: 63.027 and 73.065 tentatively identified as ethanethiol and 2-butanone/butanal. As previously highlighted by the NO^+ data, the "YES" category has a higher concentration of 2-butanone and it is probably recognised by panellists as a marker for good quality of the product. For ethanethiol the finding is counterintuitive. This sulphur compound is well known for its sulphurous vegetable-like aroma (Parker, 2014). Nevertheless it has been shown to have a positive impact on both beer and wine flavour (Liu, 2015), its higher concentration in the "YES" class was unexpected. It may be possible that this marker at low concentrations [< 10 ppbV] has a positive contribution to the quality perceived by the panel. However, more investigation is needed in this direction to clarify the contribution of this sulphur volatile compounds to the whole AMF aroma.

Table 3
Confusion matrixes of the different test sets.[a].

Predicted versus original	Reduced data (selected masses)		Raw data (all masses)	
	NO	YES	NO	YES
H_3O^+				
NO	82	18	84	16
YES	11	89	8	92
NO^+				
NO	97	3	97	3
YES	6	94	3	97
O_2^+				
NO	89	11	79	21
YES	12	88	16	84
Aggregate matrix				
NO	88	12	81	19
YES	11	89	7	93

[a] Average values were obtained by a 1000 × prediction. Classification rates are reported in %. In the case of the aggregate matrix, the all masses dataframe was obtained by selecting all mass peaks that had a concentration above 1 ppbV.

4. Conclusions

This research implemented a rapid non-invasive mass spectrometry method to classify industrial anyhdrous milk fat (AMF) samples according to the indications of an industrial sensory quality assessment. The 39 AMF samples from different suppliers were classified based on sensory analysis conducted according to the industrial partner internal protocol and according to thermal treatment. PTR/SRI-ToF-MS method was applied to screen VOC emissions of these samples and both unsupervised and supervised multivariate data analysis showed promising results in discriminating the three different classes ("YES", "NO" and "AGED").

Univariate data analysis revealed some quality markers for each class giving indications about possible key compounds that should be monitored carefully during VOC headspace analysis. The implementation of other reagent ions added information for identification of these markers and provides better classification models. Particularly, samples that underwent the thermal treatment ("AGED") had the highest concentrations of VOCs, due to thermal oxidation. The "NO" class, which contained samples with sensory defects accumulated during the different steps of the food supply chain, presented few mass peaks at significantly higher levels than the other classes which may be used as quality markers. On the other hand, "YES" samples are, in general, characterised by lower VOC release indicating that a higher concentration of VOCs penalised the sensory evaluation of the samples.

The more relevant comparison for industrial purposes, namely the one between "YES" and "NO" samples, PLS-DA showed a correct classification rate between 80 and 97%. It is reasonable to speculate that with more representative sampling and with a more accurate definition of sensory data, the volatilome analysis with the described method can be implemented profitably in industrial quality control programs.

Future studies in this direction with larger sampling, should test the reliability of our model on a larger dataset to increase the

classification rate by also selecting a pool of critical mass peaks and by further investigating the origin of the mentioned quality markers. These markers may be related to one or more stages in the food supply chain such as for example dairy cow feed, storage of milk samples and the manufacturing process. As well, more effort should be dedicated towards improving uncertainties linked to the industrial sensory evaluation, where an arbitrary score threshold was assigned to classify samples quality and an accurate definition of the evaluation error is missing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2020.104729.

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