



HAL
open science

Discrimination of spoiled beef and salmon stored under different atmospheres by an optoelectronic nose. Comparison with GC-MS measurements

Pauline Claus, Thomas Cattenoz, Sophie Landaud, Stéphane Chaillou, Anne-Claire Peron, Gwendoline Coeuret, Sami Slimani, Thierry Livache, Yann Demarigny, Daniel Picque

► To cite this version:

Pauline Claus, Thomas Cattenoz, Sophie Landaud, Stéphane Chaillou, Anne-Claire Peron, et al.. Discrimination of spoiled beef and salmon stored under different atmospheres by an optoelectronic nose. Comparison with GC-MS measurements. *Future Foods*, 2022, 5, pp.100106. 10.1016/j.fufo.2021.100106 . hal-03708769

HAL Id: hal-03708769

<https://isara.hal.science/hal-03708769v1>

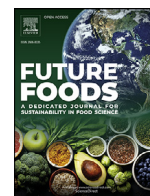
Submitted on 29 Jun 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Discrimination of spoiled beef and salmon stored under different atmospheres by an optoelectronic nose. Comparison with GC-MS measurements

Pauline Claus^a, Thomas Cattenoz^a, Sophie Landaud^a, Stéphane Chaillou^b, Anne-Claire Peron^a, Gwendoline Coeuret^a, Sami Slimani^c, Thierry Livache^c, Yann Demarigny^d, Daniel Picque^{a,*}

^a Univ Paris Saclay, UMR SayFood, INRAE, AgroParisTech, F-78850 Thiverval Grignon, France

^b Univ Paris Saclay, Micalis Institute, INRAE, AgroParisTech, F-78350 Jouy-en-Josas, France

^c Aryballe, 38000 Grenoble, France

^d ISARA Lyon, BioDyMIA, Agropole, F-69364 Lyon, France

ARTICLE INFO

Keywords:

Electronic nose
GC-MS
Beef spoilage
Salmon spoilage
Storage condition

ABSTRACT

The aim of this study was to evaluate the capacity of an electronic nose, the NeOse Pro, to assess the alteration of two food matrixes of animal origin, beef and salmon. For each matrix, two types of samples were analyzed, natural samples and simplified “diluted” samples based on meat juice and agar. Samples were inoculated with specific spoilage organisms and stored for 6 days at 8°C under different conditions: air, modified atmosphere packaging, and vacuum packaging. A non-inoculated control sample was stored at -80°C under vacuum packaging. Results of the NeOse Pro were compared with gas chromatography coupled to mass spectrophotometry analysis. For this purpose, heatmaps, principal component analysis and discriminant analysis were used. GC-MS results show that the major detected volatile organic compounds for beef stored under air are dimethyl disulfide and ethyl acetate. For salmon stored under air, it was mainly dimethyl disulfide, methyl thioacetate, acetoin and ethyl acetate that were produced. For beef and salmon NeOse Pro and GC-MS results are consistent; samples stored under air are separated from other samples.

1. Introduction

Although the consumption of vegetable proteins is increasing year by year, meat and fish are still an important source of proteins for humans. Over the last ten years, the FAO (Food and Agriculture Organization) observed that meat consumption for OECD (Organization for Economic Co-operation and Development) countries has increased by 13.9%, reaching 121,828 tons per year, whereas fish consumption remained stable for the same period at a high level of 39,253 tons per year (OCDE/FAO 2021). The main issue for consumers is having access to authentic and safe products. Quality control is a global public health issue that contributes to improving food sustainability and avoiding waste and foodborne illnesses. The main cause of spoilage in food is the proliferation of some of the microorganisms that cause changes in appearance, texture, odor and flavor in the product.

Numerous techniques exist for food quality assessment, including microbiological, sensory and physico-chemical analyses, and the development of new methods is the subject of many reviews, especially for meat (Fletcher et al., 2018) and fish (Wu et al., 2019; Prabhakar et al., 2020).

Food deterioration is characterized by the modification of organoleptic properties like the development of an unpleasant smell. This odor can be used to evaluate food freshness on the basis of several volatile organic compounds (VOCs) produced by spoilage bacteria. These VOCs are referred to chemical spoilage indicators (CSIs) (Gram and Huss, 1996).

The predominant method to study the food volatilome is gas chromatography coupled to mass spectrophotometry (GC-MS). Many studies using GC-MS have been conducted to quantify VOCs and to identify the CSIs in beef and salmon samples stored under several atmospheres (Leduc et al., 2012; Mikš-Krajník et al., 2016; Mansur et al., 2019; Kuuliala et al., 2019).

GC-MS is a reliable method of identifying and quantifying components. However, it requires expensive equipment and advanced technical requirements, and is time-consuming as well (Conti et al., 2020). Another option is the electronic nose (e-nose), a compact and user-friendly device that delivers a fast response. Moreover, e-noses use a non-destructive method and make it possible to monitor processes, making it particularly well adapted to the food-processing sector.

* Corresponding author at: Univ Paris Saclay, UMR SayFood, INRAE, AgroParisTech, F-78850 Thiverval Grignon, France
E-mail address: daniel.picque@inrae.fr (D. Picque).

E-noses are inspired by the human olfactory system. They consist of an array of gas sensors, with non-specific responses, and an appropriate pattern recognition system using the multivariate data analysis (Górska-Horczyca et al., 2016; Wu et al., 2019; Franceschelli et al., 2021). Recent reviews reporting different applications of e-noses have shown promising results for their use to evaluate meat and fish freshness (Wojnowski et al., 2017; Jia et al., 2018; Zaukuu et al., 2020). For example, the Food Sniffer electronic nose (FS) used by Ramirez et al. (2018) classifies pork meat into three categories (fresh, well cooked, spoiled) depending on storage time at 4°C. However, the spoilage assessed by sensory analysis occurred earlier than that indicated by the FS. Using a portable and MOS sensor-based e-nose system implemented with a classification algorithm (K-NN), Grassi, Benedetti, Opizzio, di Nardo, & Buratti (2019) were able to correctly classify meat and fish samples into three freshness classes defined as green-unspoiled, yellow-acceptable and red-spoiled. Based on the same classification and using a semiconductor gas sensor array, Chen et al. (2019) distinguished pork, beef and mutton samples with different storage times, in agreement with the results of the sensory evaluation. Aggregated measurements by electronic nose, computer vision and artificial tactile sensory technologies can distinguish meat samples (pork and chicken) with different freshness levels and storage times (Weng et al., 2020).

An e-nose that uses odorant binding proteins (OBPs) as sensing elements with a surface plasmon resonance imaging (SPRi) detection system has proven to be efficient for the detection of VOCs with high sensitivity and good repeatability (Hurot et al., 2019). With SPRi detection used by NeOse Pro (Aryballe), the difference of aromatic evolution of milks fermented by different *lactic acid bacteria* ratios has been highlighted (Demarigny et al., 2021).

This study seeks to go further by investigating whether NeOse Pro can detect volatile composition differences in beef or salmon stored under various conditions (air, modified atmosphere, vacuum) and inoculated with a spoilage microbiota. GC-MS is used as a reference method and to identify spoilage indicators. Data are analyzed using heatmaps, principal component analysis (PCA) and linear discriminant analysis (LDA).

2. Material and methods

2.1. Food matrix

Beef carpaccio and salmon fillet from local retailers were used to prepare natural matrix (N) and simplified matrix (S) based on diluted (1/5 times) meat juice solidified in agar. The interest of S is to have a more stable and easier to use matrix with the same characteristics as the natural one for the studies of spoilage microorganisms. N was made of raw slices of carpaccio or salmon fillet. S was made of 200 g of carpaccio or salmon fillet mixed in 1 L of water to obtain a juice. 12.5 mL of this beef or salmon juice were filter sterilized (0.2 µm) and were poured into petri dishes with the same volume of a 3% agar solution to constitute a 25 g disc of simplified beef or salmon.

Both of these N and S were inoculated at 10^3 cfu.g⁻¹ with a set of 25 specific spoilage organisms (SSOs) identified on meat and seafood products (Chaillou et al., 2015), including lactic acid bacteria, Enterobacteria and Pseudomonas. 2 slices of raw carpaccio for N and 2 discs of agar for S were put into bags with three storage conditions: vacuum packaging (VP), modified atmosphere 50% CO₂ / 50% N₂ (MAP), or air (A). Following incubation at 8°C for 6 days, 3 g of beef or salmon (N, S) were weighted and placed in 20 mL GC vials. At that time, samples were frozen at -80°C waiting for analysis. Non-inoculated controls samples (C) were prepared with beef or salmon and stored under vacuum atmosphere at -80°C. Samples were prepared in biological triplicates for each of the four storage conditions. Thus, 24 beef samples (12 N, 12 S) and 24 salmon samples (12 N, 12 S) were generated.

2.2. NeOse Pro analysis

The NeOse Pro (Aryballe, Grenoble, France) was used to evaluate flavor profiles of beef and salmon stored under different conditions. The optoelectronic nose measurement principle is based on SPRi (Brenet et al., 2018; Maho et al., 2020). Sensing materials of the NeOse Pro are short peptides fixed on a prism with a gold surface. Peptide sequences are selected for their capacity to interact with a wide range of volatile organic compounds (VOCs) rather than to their capacity to target specific ones. Therefore, each peptide will bind with VOCs present in the gas sample depending on their relative affinity. The stronger the binding reaction between a peptide and a VOC is, the greater the change of reflectivity caught by a camera from the optical system will be (Brenet et al., 2018). A pump (60 mL/min) brings the sample headspace to the sensitive material. NeOse Pro is coupled to an automatic gas sampling system (Aryballe, Grenoble, France) with 8 measurement lines. The first line, reserved to ambient air, is used to measure the baseline. Other lines are connected to 6 samples and one tracer (water in our case) for the drift correction. For each sample, the device measures the baseline for 20 s, then the sample headspace for 20 s. Next, ambient air is pumped while the signal return to baseline. The software (NeOse App, Aryballe, Grenoble, France) records via SPRi the kinetic responses of the 59 sensors which can be read on a sensorgram, and a response called signature, which is the difference between the baseline's average signal and the sample's average signal for each of the 59 sensors. This signature is representative of the affinity of analytes presents in the sample headspace and peptides coated on the prism surface. Each sample is analyzed 3 times.

The day before measurements, samples were placed at 4°C for the night. Two hours before analysis, they were heated up to 30°C. This temperature was maintained during all the measurements. Neose Pro experiments were conducted on 2 different days with 2 sets of the same samples. Since peptides as chemical sensors are likely to drift over time, a drift compensation was used as described in Maho et al. (2020).

2.3. GC-MS analysis

The most used method to evaluate the capacity to detect spoiled food is GC-MS.

In this study, the VOCs of samples were analyzed with a DHS system with a Gerstel MPS autosampler (Mülheim an der Ruhr, Denmark) and a GC-MS (7890B GC Agilent, Santa Clara, United States). Before analysis samples stored at -80°C were placed at 4°C for 16 hours. Then, they were placed in the Gerstel MPS autosampler support by the DHS system at 10°C and analyzed randomly. To generate the headspace, samples were heated at 40°C and stirred during 3 min. The headspace was purged with a flow of helium (15 min at 30 mL/min). VOCs were collected on the sorbent material (Tenax TA 2,6-diphenylene oxide polymer, Gerstel) at 30°C and dried by a helium flow (14 min at 50 mL/min). Desorption and cryofocusing of compounds were performed with a Thermal Desorption Unit connected to a Cooling Injection System (TDU-CIS Gerstel) in a solvent venting mode. Samples were heated in the TDU unit from 30°C to 270°C (60°C/min) and kept at 270°C for 7 minutes, then cryofocused in the CIS at -100°C, desorbed at 270°C (12°C/s) and held at temperature for 5 min. Then, VOCs were carried by a helium flow (1,6 mL/min) to an DB5MS capillary column (60 m x 0.32 mm x 1 µm, PEG, Agilent) in the gas chromatograph unit. The oven temperature was 10°C / 5 min, heating at 4°C/min until 130°C, heating at 20°C/min to 250°C and maintaining 250°C for 5 minutes. Analytes were then ionized in the quadrupole mass spectrometer (5977B Agilent, Santa Clara, United States) in the electron-impact mode (70 eV). Compounds were identified by matching their mass spectra with mass spectral library NIST 17 using a similarity index > 85%. To improve the results, the identification was confirmed with pure standard molecules (Sigma-Aldrich, Saint-Quentin-Fallavier, France).

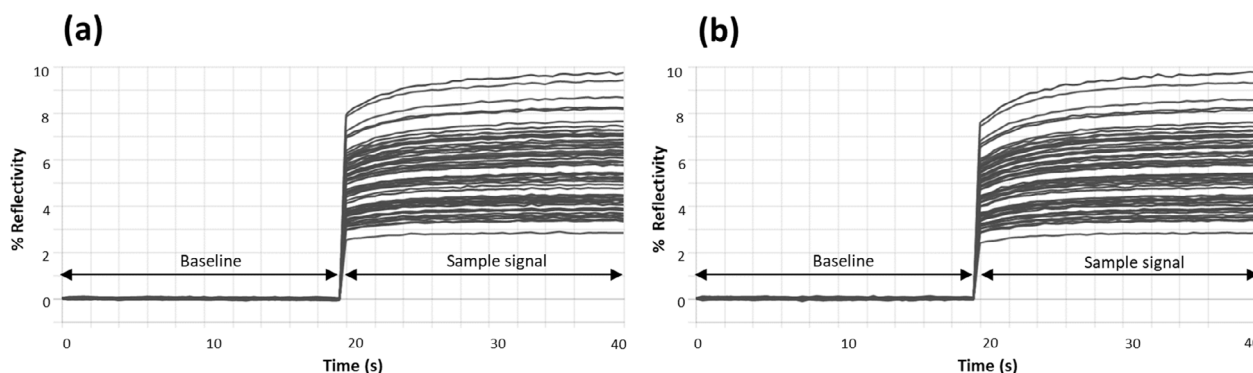


Fig. 1. NeOse Pro Sensorgrams obtained 59 sensors from the headspace analysis of beef (a) and salmon (b) C samples stored for 6 days under a vacuum atmosphere at -80°C as a function of time. Sensorgrams after exposure to air (0s – 20s) and to headspace of samples (20s – 40s).

2.4. Statistical analysis

Sample measurements were randomized in NeOse Pro and GC-MS. Data analysis was performed using heatmaps, principal component analysis (PCA) and linear discriminant analysis (LDA) with the statistical software programs XLSTAT (Addinsoft, Paris, France, 2009). For the heatmaps, an interquartile range of 0.25 was applied to remove the variables with a low variability and improve the readability. In the case of LDA methods the objects from the training set were classified using the cross-validation method with a forward variable selection. An evaluation of correctness of classification was performed using so-called confusion matrix.

3. Results and discussion

3.1. Application of NeOse Pro to evaluate flavor profiles of beef and salmon stored under different atmospheres

3.1.1. Sensor responses

Sensorgrams on Fig. 1 show typical kinetic responses of the 59 NeOse Pro sensors during the measurement of beef and salmon C samples after 6 days of storage under vacuum atmosphere at -80°C . As described by Brenet et al. (2018) and Maho et al. (2020), all the sensorgrams have the same characteristic shapes. The extremely low signals recorded during the first 20 s, are the response of the sensors under airflow and represent the baseline. When the headspace of the sample is transferred to the sensors (after 20 s), the signals quickly increase to a maximum value. Then, they remain stable at different levels during the analysis of the samples. They decrease to the baseline when the sensors are flushed with ambient air (data not shown). The steady state values depend on different binding reactions between peptides and VOCs.

Regardless of the product analyzed the normalized signatures obtained after the headspace analysis of N, S beef and salmon samples stored under different atmospheres, appear to be similar with the same shape (data not shown). However, for several sensors, the signature levels for the analysis of samples stored under air are higher than for samples stored under other conditions.

3.1.2. Multivariate analysis of Neose Pro data

A PCA was performed with the signatures of the 59 sensors for beef (Fig. 2a) and salmon (Fig. 2b) samples. The first two axes of the PCA account for 92% of the total variance for beef and 85% for salmon. In both PCAs, N (natural matrix) and S (simplified matrix) were not discriminated. The similar location of N and S samples on the PCA indicates that S is representative of N.

The beef and salmon PCAs are alike for sample location. Inoculated samples of beef and salmon stored under A were clearly discriminated from all the others on PC1, which explains about 80% and 56% of the

total variance, respectively. Non-inoculated C samples and inoculated samples stored under VP (N and S) or MAP (N and S) overlapped. Axes 3 and 4 of beef and salmon PCAs provide no better discrimination of the samples (data not shown).

Tables 1 and 2 present the variables the most strongly correlated with the axis of the PCA for beef and salmon samples. Variables are identified by numbers from 0 to 58 for each peptide that is on the golden coat of the prism. Several sensors, 3, 4, 5, 17, 25, 43 and 44, corresponding to the variables that are the most highly correlated with the positive side of PC1, are common to beef and salmon samples. Thus, these peptides that are highly and positively correlated with the first axis of the PCA are sensitive to VOCs produced for samples stored under A. Peptide 39 is common to beef and salmon but negatively correlated with PC1. This peptide is sensitive to VOCs produced for samples stored under protective atmospheres.

For the PC2, sensors 2, 16, 30 and 33 are highlighted for salmon samples and none for beef. However, no discrimination depending on the storage conditions for either beef or salmon samples is observed on the PC2 of the PCAs.

PCA results revealed the ability of NeOse Pro to detect the difference among beef and salmon samples stored under various atmospheres. In addition, cross-validated LDA was used to further assess NeOse Pro performances. The results are shown in the confusion matrix that described the number of correct and incorrect classifications for beef (Table 3) and salmon (Table 4). These tables showed that 59% of all beef samples and 62% of all salmon samples are correctly classified any storage requirements. More precisely, samples of beef and salmon stored under A were well-classified at 100% and 94% respectively. For the other classes, C, VP and MAP, the rate of well-classified samples is much lower: between 28% and 60% for beef and 44% and 72% for salmon. C, VP and MAP samples are mixed together but none of these samples were classified with those stored under A.

For both matrixes, beef and salmon, PCAs for different storage conditions showed that NeOse Pro clearly differentiates samples stored under air from samples packaged in a protective atmosphere. It does not allow the discrimination between non-inoculated controls and inoculated samples stored in VP and in MAP. This was confirmed by cross-validated LDA. These results show that the VOCs generated could be different depending on whether they were stored under air or protective atmospheres.

3.2. Application of GC-MS to evaluate flavor profiles of beef and salmon stored under different atmospheres

3.2.1. Headspace analysis of the samples

All storage conditions combined, the GC-MS detected a total amount of 52 and 84 compounds in beef and salmon samples, respectively. Amounts of VOCs released varied with the storage conditions and were

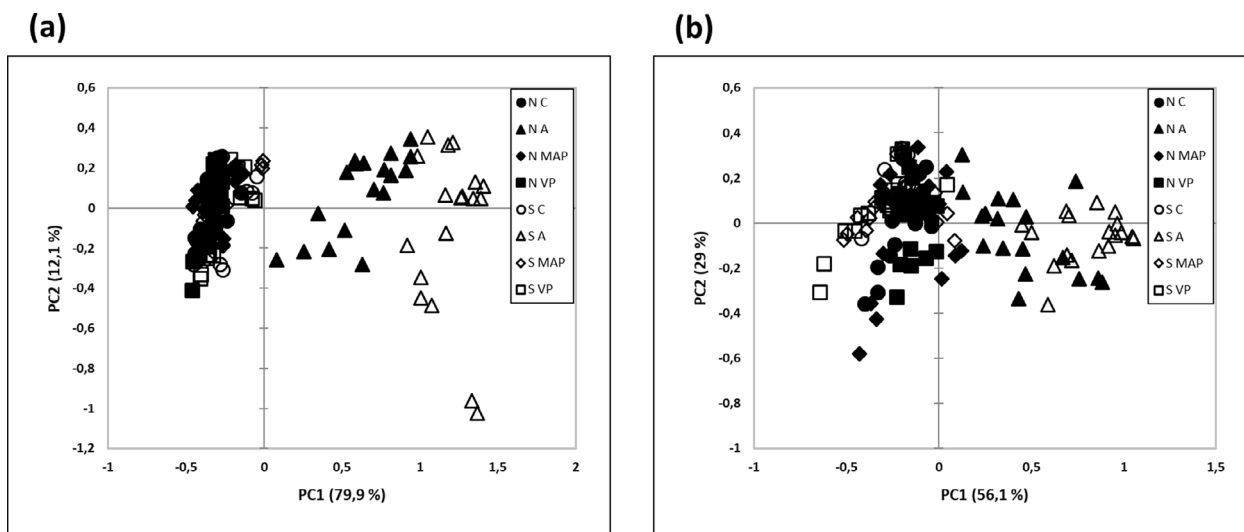


Fig. 2. Principal component analysis (PCA) score plot in dimension 1-2 for different storage conditions of beef (a) and salmon (b) samples measured by NeOse Pro. N (natural matrix); S (simplified matrix); C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

Table 1

Principal component 1, 2 analysis to highlight the contribution of variables (numbered sensors) in beef sample differentiation with NeOse Pro. Variables selected with a loading factor > 0.90 and < -0.90.

Principal component	Variance explained (%)	Total variance (%)	Most highly correlated variables	Loadings
PC1	79.9	79.9	4	0.993
			5	0.993
			44	0.991
			17	0.991
			25	0.989
			42	0.985
			19	0.983
			3	0.981
			43	0.977
			22	-0.984
			39	-0.960
			53	-0.955
			52	-0.953
PC2	12.1	92.0	-	-

Table 2

Principal component 1, 2 analysis to highlight the contribution of variables (numbered sensors) in salmon sample differentiation with NeOse Pro. Variables selected with a loading factor > 0.90 and < -0.90.

Principal component	Variance explained (%)	Total variance (%)	Most highly correlated variables	Loadings			
PC1	56.1	56.1	43	0.991			
			3	0.982			
			44	0.980			
			25	0.979			
			17	0.979			
			4	0.949			
			5	0.937			
			39	-0.920			
			21	-0.928			
			PC2	29.0	85.1	16	0.971
						2	0.940
30	-0.957						
33	-0.912						

mainly distributed into five classes: alcohols, aldehydes, ketones, esters and sulfur compounds commonly identified in meat and fish during storage (Casaburi et al., 2015; Leduc et al., 2012). Fig. 3 presents the distribution of the five class of VOCs detected by GC-MS in beef and salmon samples depending on the storage condition.

For beef (Fig. 3.a), C samples have the lowest amount of VOCs regardless of the class. Storage under A was the condition under which

the greatest amount of VOCs that can be considered as CSIs was accumulated, especially esters and sulfur compounds. Sulfur compounds are associated with unpleasant odors and can be considered as an index of spoilage (Stutz et al., 1991). Ketones were produced in greater amounts in VP and MAP.

For salmon (Fig. 3b), VOCs in C samples are either very low when compared to other conditions or absent. Like beef, samples packaged

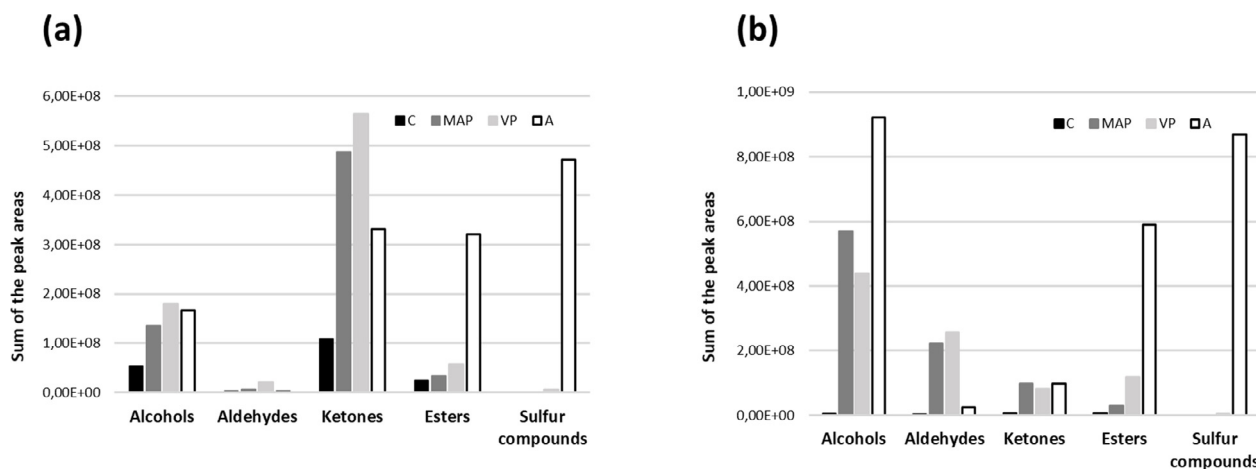


Fig. 3. Distribution of different class of VOCs (alcohols, aldehydes, ketones, esters, sulfur compounds) detected by GC-MS in beef (a) and salmon samples (b) stored under different conditions. C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

Table 3

Confusion matrix from cross validation for 4 classes corresponding to different storage conditions of beef with NeOse Pro measurements. C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

Actual class	Class size	Predicted class				% correct
		C	A	MAP	VP	
C	36	18	0	2	16	50%
A	36	0	36	0	0	100%
MAP	35	4	0	21	10	60%
VP	36	13	0	13	10	28%
Total	143	35	36	36	36	59%

Table 4

Confusion matrix from cross validation for 4 classes corresponding to different storage conditions of salmon with NeOse Pro measurements. C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

Actual class	Class size	Predicted class				% correct
		C	A	MAP	VP	
C	36	26	0	5	5	72%
A	36	1	34	1	0	94%
MAP	36	12	0	13	11	36%
VP	36	11	0	9	16	44%
Total	144	50	34	28	32	62%

under A accumulated the highest levels of VOCs known as CSIs, with alcohols, esters and sulfur compounds. Aldehydes are present in higher amount in VP and MAP samples.

3.2.2. Multivariate analysis of GC-MS data

3.2.2.1. Heatmaps analysis of beef and salmon volatile compounds. Heatmaps were generated for a rapid assessment of the variations in the contents of principal VOCs in beef (Fig. 4a) and salmon (Fig. 4b) samples under different storage conditions. A green square indicates that VOC levels were higher than their mean levels, while a red box indicates that VOC levels were lower than their mean levels.

The heatmap (Fig. 4a) classified 12 most representative VOCs and beef samples using a hierarchical cluster analysis (HCA). Two clusters were clearly defined. The first one is composed of N A and S A samples and is characterized by a higher amount of ethyl acetate, dimethyl disulfide, methyl thioacetate, 2-pentanone and 2-heptanone known as CSIs (Casaburi et al., 2015; Ercolini et al., 2011). Samples stored under a protective atmosphere {N C; S C; N MAP; S MAP; N VP; S VP} formed the second cluster. This class is defined by higher amount of 1-pentanol.

In this cluster, N C and S C were differentiated from other samples MAP and VP thanks to 3 VOCs present in higher amount: 1-pentanol, 1-octen-3-ol and acetic acid 2-phenylethyl ester. This heatmap also highlighted the high amount of acetoin in all samples.

The heatmap (Fig. 4b) classified 14 most representative VOCs and salmon samples thanks to a HCA. This heatmap distinguishes two clusters of samples. The first one {N C; S C; N MAP} is characterized by a high content of isopropyl alcohol and acetic acid 2-phenylethyl ester. This group can be divided in two sub-groups: {N C; S C} where these two molecules are higher than the other sub-group {N MAP}. The second group {S MAP; S VP; N VP; S A; N A} is mainly defined by 3-methyl-1-butanol. It can be divided in 3 sub-groups: {S MAP; S VP}, {N VP} and {S A; N A}. The sub-group {S MAP; S VP} displays high amount of 2-methyl-1-butanol, 3-methylbutanal and 2-methylbutanal. The sub-group {S A; N A} present the highest content of dimethyl disulfide and ethyl acetate which are well known CSIs (Leduc et al., 2012) and methyl thioacetate.

3.2.2.2. PCA and LDA analysis of beef and salmon volatile compounds. PCA was applied to the volatile compounds to analyze differences among the samples of beef or salmon from different storage conditions. Fig. 5a shows the PCA plot for the beef samples. The first two principal components explained about 77% of the variance. N and S samples stored under protective atmosphere (C, VP, MAP), except one N VP, were located on the positive side of the PC1 axis, which accounts for 56.5% of the variance and overlapped. N A and S A samples were dispersed on the negative side of PC1. The fragmented distribution of the air samples could be related to a wide diversity of VOCs.

The loading factors described in Table 5 for beef samples analysis make it easier to identify VOCs that contribute to the discrimination of samples. The most highly correlated VOCs are acetoin (0.979) and dimethyl disulfide (-0.837) on PC1, and ethyl acetate (0.782) on PC2. Dimethyl disulfide and acetoin contribute to the differentiation of A samples from C, VP and MAP samples. Dimethyl disulfide is one of the most common sulfur compounds found in spoiled meat during storage in air (Casaburi et al., 2015). In non-inoculated samples (C), acetoin was present, like in VP and MAP samples, but in greater quantity. It is the most commonly found ketone in meat, regardless of storage conditions. Ethyl acetate is not found in C samples and is present in other samples, with greater amounts in A. It is one of the major esters found in spoiled meat (Casaburi et al., 2015; Ercolini et al., 2011).

ACP results for salmon samples are shown in Fig. 5b. PC1 and PC2 accounted for 26.5% and 25.2% of the variance respectively (51.7% of the total variance). C samples from both N and S overlapped and formed a cluster with samples from N stored in MAP, on the negative side of the

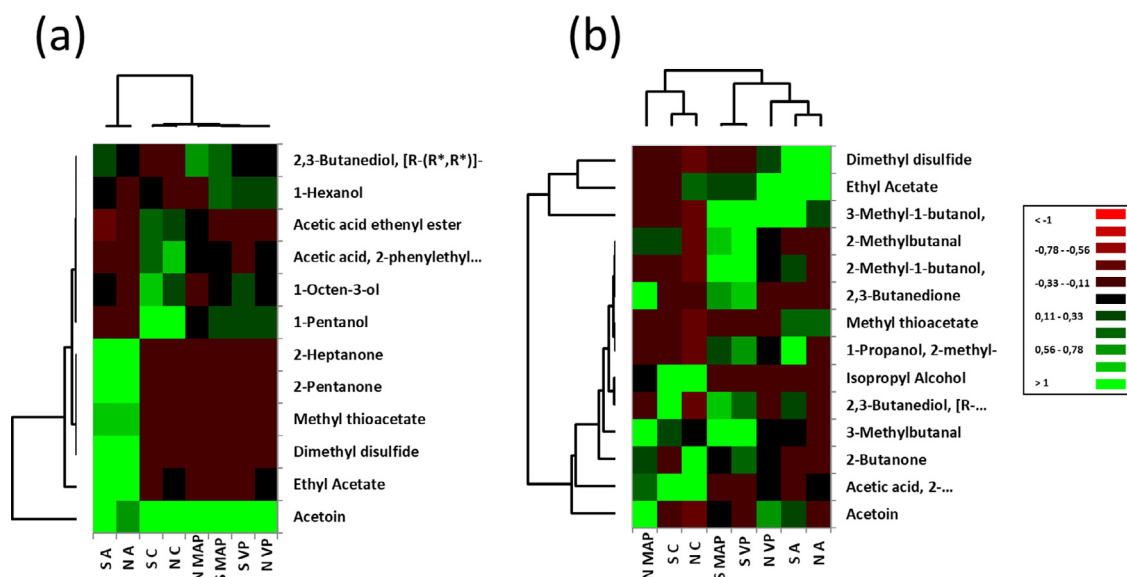


Fig. 4. Heatmaps displaying the results of hierarchical cluster analyses (HCA) conducted on both VOCs and storage conditions for beef (a) and salmon (b). Importance of VOCs varies from > 1 (highest values in green) to <-1 (lowest values in red). VOCs with an interquartile range lowest than 0.25 were removed. N (natural matrix); S (simplified matrix); C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

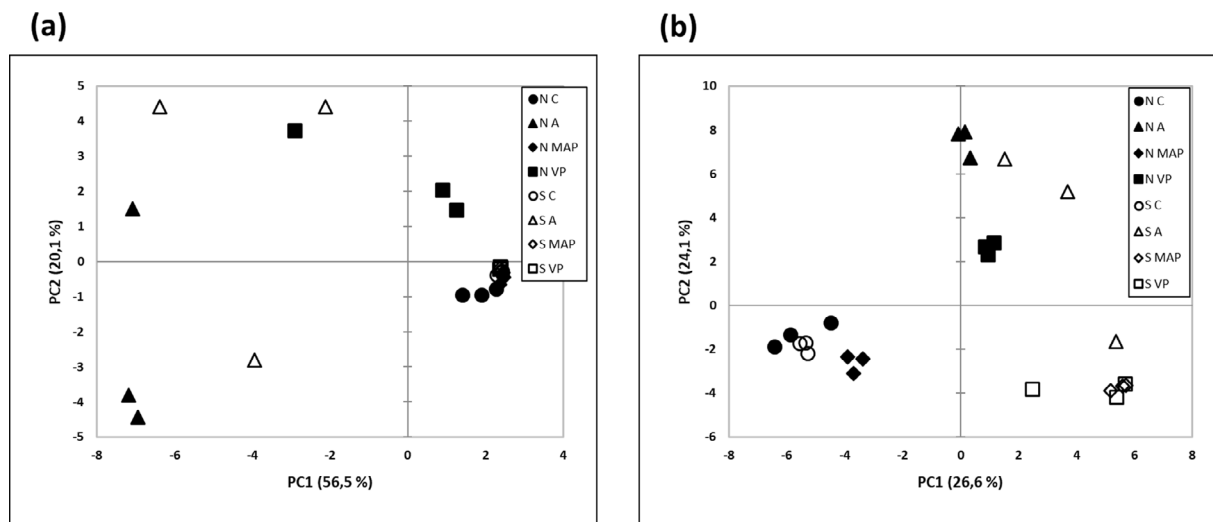


Fig. 5. Principal component analysis (PCA) score plot in dimension 1-2 for different storage conditions of beef (a) and salmon (b) samples measured by GC-MS. N (natural matrix); S (simplified matrix); C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

Table 5

Principal component 1 and 2 analysis to highlight the contribution of variables (volatile compounds) in beef sample differentiation with GC-MS. Variables selected with a loading factor > 0.70 and < -0.70.

Principal component	Variance explained (%)	Total variance (%)	Most highly correlated variables	Loadings
PC1	56.5	56.5	Acetoin Dimethyl disulfide	0.979 -0.837
PC2	20.1	76.6	Ethyl acetate	0.782

PC1 axis. S VP and S MAP samples and one S A sample formed another distinct cluster on the positive side of PC1. N VP samples were also differentiated from the others. N A and S A samples were differentiated from other samples on the PC2 axis. Since the first two axes PC1 and PC2 represent only 51.7% of the total variance, it is considered that the following axes increase the total explained variance. To reach 81.3% of the total variance, PC3 and PC4 must be considered, with 15.3% and 14.3% of the variance respectively (Table 6). Unfortunately, PC3 and

PC4 did not improve the distinction between the different storage conditions (data not shown).

The most highly correlated VOCs on PC1 are 3-methyl-1-butanol (0.934), 2-methyl-1-butanol (0.788), phenylethyl alcohol (0.755) and 2-methylbutanal (0.704) (Table 6). On PC2, methyl thioacetate (0.803) and dimethyl disulfide (0.787) are the most strongly correlated. On PC3 and PC4, the most highly correlated VOCs are acetoin (0.850) and ethyl acetate (0.805), respectively. Most of these compounds show an increase

Table 6

Principal component 1, 2, 3 and 4 analysis to highlight the contribution of variables (volatile compounds) in salmon sample differentiation with GC-MS. Variables selected with a loading factor > 0.70 and < -0.70.

Principal component	Variance explained (%)	Total variance (%)	Most highly correlated variables	Loadings
PC1	26.5	26.5	3-methyl-1-butanol	0.934
			2-methyl-1-butanol	0.788
			Phenylethyl alcohol	0.755
			2-methylbutanal	0.704
PC2	25.2	51.7	Methyl thioacetate	0.803
			Dimethyl disulfide	0.787
PC3	15.3	67.0	Acetoin	0.850
PC4	14.3	81.3	Ethyl Acetate	0.805

Table 7

Confusion matrix from cross validation for 4 classes corresponding to different storage conditions of beef with GC-MS measurements. C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

Actual class	Class size	Predicted class				% correct
		C	A	MAP	VP	
C	6	5	0	1	0	83%
A	6	0	6	0	0	100%
MAP	6	0	0	6	0	100%
VP	6	0	1	2	3	50%
Total	24	5	7	9	3	83%

Table 8

Confusion matrix from cross validation for 4 classes corresponding to different storage conditions of salmon with GC-MS measurements. C (control); A (air); MAP (modified atmosphere); VP (vacuum packaging).

Actual class	Class size	Predicted class				% correct
		C	A	MAP	VP	
C	6	5	0	1	0	83%
A	6	0	6	0	0	100%
MAP	6	0	0	5	1	83%
VP	6	0	0	1	5	83%
Total	24	5	6	7	6	88%

during storage, and some studies suggest that certain ones are spoilage markers for fish. This is the case of 3-methyl-1-butanol for ice-stored fish in VP and under A (Leduc et al., 2012; Soncin et al., 2009) and raw Atlantic salmon stored under A (Mikš-Krajnik et al., 2016), 2-methyl-1-butanol in VP (Jørgensen et al., 2001; Jónsdóttir et al., 2008) and 2-methylbutanal for ice-stored fish in VP or under A (Leduc et al., 2012; Soncin et al., 2009). In our study, these three compounds contributed to the differentiation on PC1 of S VP, S MAP samples and 1 S A sample from the other ones. Dimethyl disulfide made it possible to distinguish A samples from C samples on PC2 in this study. It is also considered as a CSI for ice-stored fish in VP and under A (Leduc et al., 2012; Alasalvar et al., 2005). On PC2, methyl thioacetate a molecule with an unpleasant odor contributes to the differentiation of A samples as well.

GC-MS showed differences between storage conditions both for beef and salmon. The most important distinction appeared between samples stored under air and non-inoculated samples (C). Among the detected VOCs that differentiate samples stored under A from the others, dimethyl disulfide is common for both beef and salmon samples.

The matrix confusion for the cross-validated LDA of beef samples (Table 7) and salmon samples (Table 8) showed that classifications were correct with an 83% and 88% rate respectively. All of the beef and salmon samples stored under A were well classified, as were beef samples in MAP. For the other classes, the rate of correct classification reached 83% except for beef samples stored in VP (50%). These results confirmed the PCA analysis and the specificity of samples stored under A.

4. Conclusions

We have demonstrated that NeOse Pro is a new analytical tool that is particularly interesting for the assessment of food matrices spoiled by microorganisms. NeOse Pro allowed the differentiation of salmon and beef samples stored under air from those stored under protective atmospheres. Several peptides are responsible for this differentiation. In agreement with NeOse Pro results, GC-MS showed the same differentiation and detected greater amounts of VOCs in samples stored under air, such as dimethyl disulfide, which can be found in both beef and salmon samples. The signature supplied by NeOse Pro is sufficient to identify spoiled food. It facilitates food quality assessment by being an easily transported device that provides a rapid answer. Based on these findings, future developments will focus on the optimization of the NeOse Pro sensor array by designing and incorporating alternative sequences of the identified peptides of interest and by removing the non-informative ones.

Funding

This work was supported by the program “Programme d’investissements d’avenir” NEOSE F3 PIA3 project (France Agrimer, BPI France).

Declaration of interest

None

Acknowledgements

We thank G. WAGMAN (Sauve, France) for her editorial advice on the English version of this article.

References

- Alasalvar, C., Taylor, K.D.A., Shahidi, F., 2005. Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis/gas chromatography–mass spectrometry. *J. Agric. Food Chem.* 53 (7), 2616–2622. doi:10.1021/jf0483826.
- Brenet, S., John-Herpin, A., Gallat, F.X., Musnier, B., Buhot, A., Herrier, C., Rousselle, T., Livache, T., Hou, Y., 2018. Highly-selective optoelectronic nose based on surface plasmon resonance imaging for sensing volatile organic compounds. *Anal. Chem.* 90 (16), 9879–9887. doi:10.1021/acs.analchem.8b02036.
- Casaburi, A., Piombino, P., Nychas, G.-J., Villani, F., Ercolini, D., 2015. Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiol.* 45, 83–102. doi:10.1016/j.fm.2014.02.002.
- Chaillou, S., Chaulot-Talmon, A., Caekebeke, H., Cardinal, M., Christians, S., Denis, C., Hélène Desmonts, M., Dousset, X., Feurer, C., Hamon, E., Joffraud, J.-J., La Carbone, S., Leroy, F., Leroy, S., Lorre, S., Macé, S., Pilet, M.-F., Prévost, H., Rivollier, M., ... Champomier-Vergès, M.-C., 2015. Origin and ecological selection of core and food-specific bacterial communities associated with meat and seafood spoilage. *ISME J.* 9 (5), 1105–1118. doi:10.1038/ismej.2014.202.
- Chen, J., Gu, J., Zhang, R., Mao, Y., Tian, S., 2019. Freshness evaluation of three kinds of meats based on the electronic nose. *Sensors* 19 (3), 605. doi:10.3390/s19030605.
- Conti, C., Guarino, M., Bacenetti, J., 2020. Measurements techniques and models to assess odor annoyance: A review. *Environ. Int.* 134, 105261. doi:10.1016/j.envint.2019.105261.

- Demarigny, Y., Legrand, E., Sanchez, J., Hallier, A., Laurent, N., Slimani, S., Livache, T., Picque, D., 2021. Utilisation of a portable electronic nose, NeOse Pro, to follow the microbial fermentation of a yoghurt. *Food Nutr. Sci.* 12 (01), 90–105. doi:10.4236/fns.2021.121008.
- Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., Vernocchi, P., La Storia, A., Laghi, L., Mauriello, G., Guerzoni, M.E., Villani, F., 2011. Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. *Appl. Environ. Microbiol.* 77 (20), 7372–7381. doi:10.1128/AEM.05521-11.
- Fletcher, B., Mullane, K., Platts, P., Todd, E., Power, A., Roberts, J., Chapman, J., Cozzolino, D., Chandra, S., 2018. Advances in meat spoilage detection: a short focus on rapid methods and technologies. *CyTA - J. Food* 16 (1), 1037–1044. doi:10.1080/19476337.2018.1525432.
- Franceschelli, L., Berardinelli, A., Dabbou, S., Ragni, L., Tartagni, M., 2021. Sensing technology for fish freshness and safety: a review. *Sensors* 21 (4), 1373. doi:10.3390/s21041373.
- Górska-Horczyzak, E., Guzek, D., Mołęda, Z., Wojtasik-Kalinowska, I., Brodowska, M., Wierzbicka, A., 2016. Applications of electronic noses in meat analysis. *Food Sci. Technol.* 36 (3), 389–395. doi:10.1590/1678-457X.03615.
- Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish products. *Int. J. Food Microbiol.* 33 (1), 121–137. doi:10.1016/0168-1605(96)01134-8.
- Grassi, Benedetti, Opizzio, Nardo, Buratti, 2019. Meat and fish freshness assessment by a portable and simplified electronic nose system (Mastersense). *Sensors* 19 (14), 3225. doi:10.3390/s19143225.
- Hurot, C., Brenet, S., Buhot, A., Barou, E., Belloir, C., Briand, L., Hou, Y., 2019. Highly sensitive olfactory biosensors for the detection of volatile organic compounds by surface plasmon resonance imaging. *Biosens. Bioelectron.* 123, 230–236. doi:10.1016/j.bios.2018.08.072.
- Jia, W., Liang, G., Wang, Y., Wang, J., 2018. Electronic noses as a powerful tool for assessing meat quality: a mini review. *Food Anal. Methods* 11 (10), 2916–2924. doi:10.1007/s12161-018-1283-1.
- Jónsdóttir, R., Ólafsdóttir, G., Chanie, E., Haugen, J.-E., 2008. Volatile compounds suitable for rapid detection as quality indicators of cold smoked salmon (*Salmo salar*). *Food Chem.* 109 (1), 184–195. doi:10.1016/j.foodchem.2007.12.006.
- Jørgensen, L.V., Huss, H.H., Dalgaard, P., 2001. Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar*) analyzed by GC-MS and multivariate regression. *J. Agric. Food Chem.* 49 (5), 2376–2381. doi:10.1021/jf0009908.
- Kuuliala, L., Sader, M., Solimeo, A., Pérez-Fernández, R., Vanderroost, M., De Baets, B., De Meulenaer, B., Ragaert, P., Devlieghere, F., 2019. Spoilage evaluation of raw Atlantic salmon (*Salmo salar*) stored under modified atmospheres by multivariate statistics and augmented ordinal regression. *Int. J. Food Microbiol.* 303, 46–57. doi:10.1016/j.ijfoodmicro.2019.04.011.
- Leduc, F., Krzewinski, F., Le Fur, B., N'Guessan, A., Malle, P., Kol, O., Duflos, G., 2012. Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS: Differentiation of fresh and frozen/thawed fish by SPME/GC/MS. *J. Sci. Food Agric.* 92 (12), 2560–2568. doi:10.1002/jsfa.5673.
- Mikš-Krajnik, M., Yoon, Y.-J., Ukuku, D.O., Yuk, H.-G., 2016. Volatile chemical spoilage indexes of raw Atlantic salmon (*Salmo salar*) stored under aerobic condition in relation to microbiological and sensory shelf lives. *Food Microbiol.* 53, 182–191. doi:10.1016/j.fm.2015.10.001.
- Maho, P., Herrier, C., Livache, T., Rolland, G., Comon, P., Barthelmé, S., 2020. Reliable chiral recognition with an optoelectronic nose. *Biosens. Bioelectron.* 159, 112183. doi:10.1016/j.bios.2020.112183.
- Mansur, A.R., Seo, D.-H., Song, E.-J., Song, N.-E., Hwang, S.H., Yoo, M., Nam, T.G., 2019. Identifying potential spoilage markers in beef stored in chilled air or vacuum packaging by HS-SPME-GC-TOF/MS coupled with multivariate analysis. *LWT* 112, 108256. doi:10.1016/j.lwt.2019.108256.
- OCDE/FAO, 2021. « Perspectives agricoles de l'OCDE et de la FAO (Édition 2020) ». Statistiques agricoles de l'OCDE doi:10.1787/aa2efdd2-fr, (base de données)(data retrieved on June 18, 2021).
- Prabhakar, P.K., Vatsa, S., Srivastav, P.P., Pathak, S.S., 2020. A comprehensive review on freshness of fish and assessment: analytical methods and recent innovations. *Food Res. Int.* 133, 109157. doi:10.1016/j.foodres.2020.109157.
- Soncin, S., Chiesa, L.M., Panseri, S., Biondi, P., Cantoni, C., 2009. Determination of volatile compounds of precooked prawn (*Penaeus vannamei*) and cultured gilthead sea bream (*Sparus aurata*) stored in ice as possible spoilage markers using solid phase microextraction and gas chromatography/mass spectrometry: volatiles of ice-stored prawn and sea bream as spoilage markers. *J. Sci. Food Agric.* 89 (3), 436–442. doi:10.1002/jsfa.3466.
- Stutz, H.K., Silverman, G.J., Angelini, P., Levin, R.E., 1991. Bacteria and volatile compounds associated with ground beef spoilage. *J. Food Sci.* 56 (5), 1147–1153. doi:10.1111/j.1365-2621.1991.tb04721.x.
- Weng, X., Luan, X., Kong, C., Chang, Z., Li, Y., Zhang, S., Al-Majeed, S., Xiao, Y., 2020. A comprehensive method for assessing meat freshness using fusing electronic nose, computer vision, and artificial tactile technologies. *J. Sensors* 2020, 1–14. doi:10.1155/2020/8838535.
- Wojnowski, W., Majchrzak, T., Dymerski, T., Gębicki, J., Namieśnik, J., 2017. Electronic noses: Powerful tools in meat quality assessment. *Meat Sci.* 131, 119–131. doi:10.1016/j.meatsci.2017.04.240.
- Wu, L., Pu, H., Sun, D.-W., 2019. Novel techniques for evaluating freshness quality attributes of fish: A review of recent developments. *Trends Food Sci. Technol.* 83, 259–273. doi:10.1016/j.tifs.2018.12.002.
- Zaukuu, J.L.Z., Bazar, G., Gillay, Z., Kovacs, Z., 2020. Emerging trends of advanced sensor based instruments for meat, poultry and fish quality—a review. *Crit. Rev. Food Sci. Nutr.* 60 (20), 3443–3460. doi:10.1080/10408398.2019.1691972.