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A NMR-BASED METABOLOMICS APPROACH FOR REDUCING FOOD LOSSES: THE EXAMPLE OF MINCED PORK MEAT.

Emilie Cauchie [1 *], Nicolas Korsak [1], Justine Leenders [2 *], Ghislain Baré [1], Assia Tahiri [1], Laurent Delhalle [1], Pascal de Tullio [2] and Daube Georges [1]

[1] University of Liège, Fundamental and Applied Research for Animal & Health (FARAH), Faculty of Veterinary Medicine, Food Science Department, Quartier Vallée, Avenue de Cureghem 10, B-4000 Liège, Belgium.

[2] University of Liège, Center for Interdisciplinary Research on Medicines (CIRM), Metabolomics group, Quartier Hôpital, Avenue Hippocrate 15, B-4000 Liège, Belgium

[*] Corresponding authors: ecauchie@uliege.be and jleenders@uliege.be

ABSTRACT

In Europe, the losses of initial meat production represent 20% and more than half of this occurs at animal production, slaughtering, processing and distribution step. In order to control food waste, studies have highlighted the importance of monitoring the microbial diversity of food products because spoilage by bacteria that contaminate the food matrix is a major issue. As such, the combination of metabolomics data with other complementary approaches (classical microbiology and quality parameters) can gives the opportunity to gain deeper insights into and have a better comprehension of the spoilage mechanisms. The aim of the current study was to assess meat spoilage through the evolution of bacterial counts and changes in the metabolic profile of minced pork meat using Nuclear Magnetic Resonance (NMR) based metabolomics. Microbiological assessment, pH measurements, gas composition and metabolomics analysis were carried out in meat samples stored under food wrap and under modified atmosphere packaging (70% O_2 – 30% CO_2) at 4, 8 and 12°C during 13 days. All samples were irradiated and then inoculated separately with three dominant bacterial species: *Brochothrix thermosphacta*, *Leuconostoc gelidum* and *Pseudomonas fragi*. For all conditions, non-inoculated samples were also stored. Analysis were carried out at day 0 and at day 13 for metabolomics analysis, and each day for all others measurements. The multivariate analysis (PLS-DA) reveals a clear discrimination between: (i) the non-inoculated product at day 0 and at day 13, (ii) the inoculated and non-inoculated samples, (iii) the type of bacterium, and (iv) the packaging conditions. It can be observed that the type of bacterium inoculated had a higher impact on the metabolome than that the packaging conditions. Moreover, some metabolites are significantly increased: acetate and glycerol for *B. thermosphacta*, betaine and lactate for *L. gelidum*, threonine and glycine for *P. fragi*. Exploration of the correlations of NMR-based metabolomics results with others microbial parameters suggested their use as possible spoilage tool to provide information on minced pork meat spoilage and to follow intrinsically the evolution of the metabolomics pattern linked to a specific bacterium in a complex bacterial ecosystem.

KEYWORDS

Meat spoilage, NMR metabolomics, *Brochothrix thermosphacta*, *Pseudomonas fragi*, *Leuconostoc gelidum*.

1 INTRODUCTION

Among the reasons for food loss and waste, spoilage by bacteria that contaminate the food matrix and are able to develop during transformation steps and storage is a major issue (Lipsinki *et al.*, 2013; Remenant *et al.*, 2015). It is well established that spoilage of meat is the result of decomposition and formation of metabolites caused by the growth and enzymatic activity of microorganisms (Argyri *et al.*, 2015), highlighting the importance of managing the quality of food products. New "omics" approaches and more specifically metabolomics, which deals with the study of the metabolites profiles of samples, is a relatively new investigate tool in food science. Recently, application of NMR spectroscopy coupled with multivariate analysis has been used to obtain metabolite profiling of various kinds of food including salmon, meat, honey, milk, olive oil, wine, tea and others plants (Jung *et al.*, 2010).

NMR spectroscopy and more specifically proton NMR is, together with mass spectrometry, the most widely used analytical platform for metabolomics analysis. It has proved to be an attractive technique offering in a single experiment an overview of a wide range of compounds present in a matrix by detecting all ^{1}H containing metabolites with concentrations above tens of micromolar level (Chen *et al.*, 2016; Yuan *et al.*, 2017). NMR-based metabolomics can be potentially useful for identifying variation between the metabolic profiles linked to typical processing and storage conditions, and can be used to identify specific fingerprinting that could be correlated with the samples status (Boffo *et al.*, 2012; Charlton *et al.*, 2002). However, among all these studies only a few have been performed on foods (Piras *et al.*, 2013), and particularly on meat (Castejon *et al.*, 2015; Ritota *et al.*, 2012). Although water content of meat and abundance of nutrients available on the surface make it one of the most perishable foods (Ercolini *et al.*, 2011), no metabolite profiles from inoculated meat by putatively spoilage microorganisms have been reported to date (Zanardi *et al.*, 2015). Then, it is expected that the combination of metabolomics data with other complementary approaches (classical microbiology and quality parameters) can gives the opportunity to gain deeper insights into, and have a better comprehension of the spoilage mechanisms (Consonni and Cagliani, 2008; Mannina *et al.*, 2012).

According to this, this work aims at studying by a NMR-based metabolomics approach the changes in metabolites profiles of minced pork meat during shelf life product and meat spoilage. For this purpose, non-inoculated and inoculated samples, respectively with *Brochothrix thermosphacta*, *Pseudomonas fragi* and *Leuconostoc gelidum* were collected, considered as one of commonly specific spoilage organism (SSO) (Miks-Krajnik *et al.*, 2016). Metabolites profiling were then measured and analysed according to modifications of some environmental parameters of storage (temperature and food packaging). These parameters are important factors that can influence the biochemical components of samples. The metabolomics data were compared with others classical methods used to follow the bacterial evolution in complex ecosystem (classical microbiology, pH values and gas composition measurements).

2 MATERIAL AND METHODS

2.1 Food samples and selection of bacterial isolates

The isolates used in this study were previously isolated from Belgian minced pork meat (three batches from four producers) at the end of their use-by date by 16S rRNA metagenetics and bacterial counts by classical microbiology. Three of the natural predominant bacteria isolated at the end of the shelf life, represented more than 50% of the natural microbiota, were identified by sequencing of their 16S rRNA genes and used for the challenge-tests: *Brochothrix thermosphacta* (MM008), *Leuconostoc gelidum* (MM045) and *Pseudomonas fragi* (*Pseudomonas fragi* MM014 and MM015). Bacterial isolates were stored at −80°C in nutrient broth with 30% glycerol as a cryoprotective agent. Before use, isolates were transferred from the −80°C culture collection to Brain Heart Infusion (BHI) broth for 48 h at 22°C. The cultures were incubated overnight at 4°C before inoculation.

Fresh minced pork meat packed under air with a food wrap film were obtained from a local Belgian manufacturer. The water activity of this product was 0.98 ± 0.02 and the pH value was 5.80 ± 0.02. Food samples were irradiated by gamma irradiation at 10 kGy (Sterigenics, Fleurus, Belgium) and were

stored until used at -20°C.

2.2 Challenge-tests

The products were inoculated, in triplicate, by adding sterile water containing each individually of the three bacterial isolates with the goal of reaching an approximatively global concentration of 3.00 log colony forming units (log CFU/g on the product) (n=465). The samples were mixed by a Kenwood mixer (Kenwood Belgium, Mechelen, Belgium) for 2 min in speed 2. Non-inoculated control samples were homogenized by adding the same quantity of sterile water only, in triplicate (n=36). Minced pork meat was then packed (50 g) in a tray (PP/EVOH/PP) under modified atmosphere (MAP, CO_2 30% / O_2 70%) (Olympia V/G, Technovac, Italy) and under food wrap packing (FW) (cling film). For this study, a short 13 days shelf life was evaluated for the minced pork meat. Inoculated samples were stored at different constant temperatures of 4°C (± 1°C), 8°C (± 1°C) and 12°C (± 1°C) in temperature-controlled incubators. Control samples were only stored at the first day of inoculation (day 0) and at day 13.

2.3 Conventional microbiological method

Each day during the 13-days storage period, 25 g of product were put into a Stomacher bag with a mesh screen liner (80 µm pore size) (Biomérieux, Basingstoke, England, ref 80015) under aseptic conditions. Physiological water (225 mL) was automatically added to each bag (Dilumat, Biomérieux, Belgium) and the samples were homogenized for 2 min in a Stomacher (Bagmixer, Interscience, France). From this primary suspension, decimal dilutions in peptone water (1 g/L peptone, 8.5 g/L sodium chloride) were prepared for microbiological analysis and 0.1 mL aliquots of the appropriate dilutions were plated onto media for each analysis in duplicate (Spiral plater, DW Scientific, England). A total count was made on Plate Count Agar (PCA) at 22°C for 48 h for the psychrotrophic aerobic plate count (PAPC), using the modified method specified by the International Organization for Standardization [ISO (2013, ISO 4833-2)].

2.4 Samples pH measurements and gas composition values

The pH of the homogenized samples (5 g in 45 ml of KCl) was measured with a pH meter (Knick 765 Calimatic, Allemagne) at day 0 and day 13. For all samples stored in modified atmosphere packaging measurements of the composition in gas was monitored daily until day 13 (CheckMate 3, Dansensor, France). Using Excel, t-student test was used to evaluate statistical differences between samples measurements, all tests were considered as significant for a p-value of < 0.05.

2.4 Samples preparation for NMR analysis

^{1}H-NMR analysis of minced pork meat samples was realized at day 0 and day 13, in five repetitions (n=240), for inoculated and non-inoculated samples. 100 mg of each meat samples were reconstituted in an eppendorf tube by adding 500 µl D_2O (DPB, pH 7.4). The mixture was homogenized with a vortex during 1 min and then centrifugated 2x30 sec at 5000 rpm. 300 µl of D_2O were added and the mixture was homogenizing with a vortex 1 min and then leave for 15 min. A last centrifugation step during 15 min at 13000 rpm was performed and the mixture was transferred to a NMR tube for the analysis of the supernatant (650 µl) by ^{1}H-NMR.

2.4 NMR measurements

All samples were recorded at 298 K on a Bruker Avance spectrometer operating at 500 MHz for the proton signal acquisition. The instrument was equipped with a 5 mm TCI cryoprobe with a Z-gradient. Maleic acid was used as internal standard for quantification and trimethylsilyl-3-propionic acid-*d*4 (TMSP) for the zero calibration. 650 µl of meat supernatant (prepared as described before) were supplemented with 100 µl of deuterated phosphate buffer, 100 µl of a 5 mM solution of maleic acid and 5 µl of a 10 mg/ml TMSP solution. ^{1}H-NMR spectra were acquired using a CPMG relaxation-editing sequence with presaturation for meat samples. The CPMG experiment used a RD-90-(t-180-t)n-sequence with a relaxation delay (RD) of 2 s, a spin echo delay (t) of 400 ms and the number of loops (n) equal to 80. The water suppression pulse was placed during the relaxation delay (RD). The number of transients was typically 32 and a number of 4 dummy scans was chosen. The data were processed with the Bruker Topspin 3.2 software with a standard parameter set. Phase and baseline corrections were performed manually over the entire range of the spectra and the δ scale was calibrated to 0 ppm using the internal standard TMSP.

2.4 Multivariate analysis

For statistical analysis, optimized ^{1}H-NMR spectra were automatically baseline-corrected and reduced to ASCII files using AMIX software (version 3.9.14; Bruker). The spectral intensities were normalized to total intensities and reduced to integrated regions of equal width (0.04 ppm) corresponding to the 0.5–10.00 ppm region. Because of the residual signals of water and maleic acid, regions between 4.7 and 5 ppm (water signal) and 5.6–6.2 ppm (maleic acid signal) were removed before analysis. The reduced and normalized NMR spectral data were imported into SIMCA (version 13.0.3, Umetrics AB, Umea Sweden). Pareto scaling was applied to bucket tables and discriminant analysis (DA) such as PCA (Principal Component Analysis), PLS-DA (Partial Least Squares Discriminant Analysis), OPLS-DA (orthogonal partial least squares discriminant analysis) and PLS (Partial Least Square) regression were performed. SIMCA was used to generate all PCA, PLS, PLS-DA, and OPLS-DA models and plots. PCA was only used to detect possible outliers and determine intrinsic clusters within the data set, while PLS-DA maximized the separation and OPLS-DA facilitated the graphic visualization of differences and similarities between groups. The quality of OPLS-DA models was determined by the goodness of fit (R^2) and the predictability was calculated on the basis of the fraction correctly predicted in one-seventh cross-validation (Q^2).

2.4 Metabolite identification

From PLS-DA loading plots, metabolites with higher loadings were identified. Signals with values of Variable Importance in Projection (VIP) higher than 1 were considered as significant, and further validated using t-test with Metaboanalyst (http://www.metaboanalyst.ca). Metabolite identification was next performed using the open-access database NMR suite 8.1 (Chenomx inc., Edmonton, Canada), the free web-based tool HMDB (http://www.hmdb.ca) and tables. Each metabolite identified was finally confirmed by performing peak correlation plots from 2D-NMR spectra (COSY and HSQC)

3 RESULTS

3.1 Microbial counts

Tables 1 and 2 show the PCA results from challenge tests at different temperature (4, 8 and 12°C) and for the two packaging (MAP and FW). As expected, the storage temperature and the packaging conditions have a strong impact on the bacterial evolutions. At 8°C, *Pseudomonas fragi* reach approximatively 10.00 and 8.30 log CFU/g in FW and MAP, respectively. A high growth rate and a more rapidly reached stationary phase are also correlated to the FW packaging and the highest storage temperatures. No bacterial growth is observed on PCA for the control samples (limit detection < 3.00 log CFU/g, results not shown).

Table 1: Microbiological counts for challenge tests in food wrap (FW) packaging, at different temperature, for a 13-days shelf life.

Days	Bacterial species		
	Brochothrix thermosphacta	***Pseudomonas fragi***	***Leuconostoc gelidum***
	4°C		
0	3.84±0.03	3.15±0.59	4.00±0.02
1	3.08±0.10	3.43±0.11	4.07±0.01
2	3.76±0.07	4.52±0.23	4.38±0.01
3	4.54±0.12	5.64±0.19	4.61±0.12
4	- [a]	- [a]	- [a]
5	- [a]	- [a]	- [a]
6	7.24±0.11	- [a]	6.17±0.05
7	7.74±0.17	9.45±0.13	- [a]
8	7.63±0.10	9.51±0.07	- [a]
9	8.17±0.33	- [a]	- [a]
10	7.68±0.15	9.90±0.29	8.62±0.09
11	- [a]	- [a]	- [a]
12	- [a]	- [a]	- [a]
13	7.90±0.15	10.21±0.03	8.42±0.06
	8°C		
0	3.84±0.03	3.15±0.59	4.00±0.02
1	6.76±0.04	3.86±0.17	4.58±0.08
2	7.49±0.11	5.36±0.03	5.84±0.02
3	8.25±0.07	7.69±0.17	- [a]
4	8.51±0.10	9.04±0.05	7.57±0.10
5	8.58±0.06	9.67±0.03	- [a]
6	8.85±0.02	- [a]	8.61±0.13
7	8.77±0.15	9.62±0.15	- [a]
8	9.05±0.03	10.34±0.24	8.73±0.07
9	8.79±0.21	10.39±0.40	- [a]
10	- [a]	10.11±0.28	8.84±0.09
11	- [a]	- [a]	- [a]
12	- [a]	- [a]	- [a]
13	9.00±0.01	10.15±0.17	8.77±0.30
	12°C		
0	3.84±0.03	3.15±0.59	4.00±0.02
1	7.68±0.08	4.93±0.15	5.38±0.01
2	8.29±0.13	- [a]	6.84±0.13
3	8.66±0.04	9.81±0.04	8.35±0.09
4	8.99±0.09	9.85±0.29	7.56±0.01
5	9.01±0.23	9.95±0.34	- [a]
6	9.11±0.10	10.15±0.82	8.64±0.13
7	8.81±0.28	10.26±0.08	- [a]
8	9.03±0.03	10.14±0.10	- [a]
9	8.91±0.16	- [a]	- [a]
10	- [a]	9.87±0.19	8.82±0.23
11	- [a]	- [a]	- [a]
12	- [a]	- [a]	- [a]
13	9.27±0.08	9.80±0.42	8.62±0.18

[a] no analysis performed for the day.

Table 2: Microbiological counts for challenge tests in modified atmosphere (MAP) packaging, at different temperature, for a 13-days shelf life.

Days	Bacterial species		
	Brochothrix thermosphacta	***Pseudomonas fragi***	***Leuconostoc gelidum***
	4°C		
0	3.84±0.03	3.15±0.59	4.00±0.02
1	- [a]	- [a]	4.18±0.09
2	- [a]	3.48±0.06	- [a]
3	2.17±0.30	- [a]	- [a]
4	- [a]	- [a]	6.31±0.17
5	- [a]	3.90±0.11	- [a]
6	4.11±0.01	4.87±0.34	6.84±0.06
7	4.01±0.14	4.55±0.12	7.85±0.01
8	4.35±0.03	- [a]	- [a]
9	5.24±0.05	- [a]	7.78±0.21
10	4.99±0.12	- [a]	- [a]
11	- [a]	- [a]	- [a]
12	- [a]	4.73±0.01	8.00±0.10
13	5.43±0.06	4.90±0.01	8.39±0.12
	8°C		
0	3.84±0.03	3.15±0.59	4.00±0.02
1	- [a]	3.52±0.01	4.75±0.03
2	5.88±0.10	4.16±0.05	- [a]
3	6.11±0.11	- [a]	- [a]
4	7.11±0.02	- [a]	8.06±0.01
5	7.86±0.10	5.41±0.08	- [a]
6	8.21±0.04	6.33±0.07	8.38±0.05
7	8.43±0.11	6.52±0.14	8.49±0.16
8	8.43±0.16	- [a]	- [a]
9	8.41±0.10	6.59±0.17	8.85±0.01
10	8.38±0.16	- [a]	- [a]
11	- [a]	- [a]	- [a]
12	7.86±0.07	7.83±0.13	- [a]
13	8.76±0.03	8.37±0.08	8.75±0.19
	12°C		
0	3.84±0.03	3.15±0.59	4.00±0.02
1	- [a]	4.47±0.07	8.32±0.15
2	7.10±0.04	6.08±0.03	7.28±0.01
3	7.76±0.23	- [a]	- [a]
4	8.35±0.04	- [a]	8.35±0.06
5	8.58±0.06	- [a]	- [a]
6	8.40±0.12	9.42±0.28	8.36±0.09
7	8.44±0.07	9.58±0.23	8.64±0.10
8	8.32±0.03	- [a]	- [a]
9	9.16±0.08	9.80±0.41	8.89±0.07
10	8.67±0.40	- [a]	- [a]
11	- [a]	- [a]	- [a]
12	8.83±0.02	9.87±0.06	- [a]
13	8.71±0.06	9.85±0.14	8.87±0.11

[a] no analysis performed for the day.

3.2 pH and gas measurements

Figure 1 shows the comparison between pH measurements for control and inoculated samples at day 13. A significant increase of pH is only observed for *Pseudomonas fragi*, which reach a higher pH value at day 13 (7.54 ± 0.76, n=5) compared to control samples (5.79 ± 0.05, n=10). There were no differences of pH values between FW and MAP conditions for control and inoculated samples.

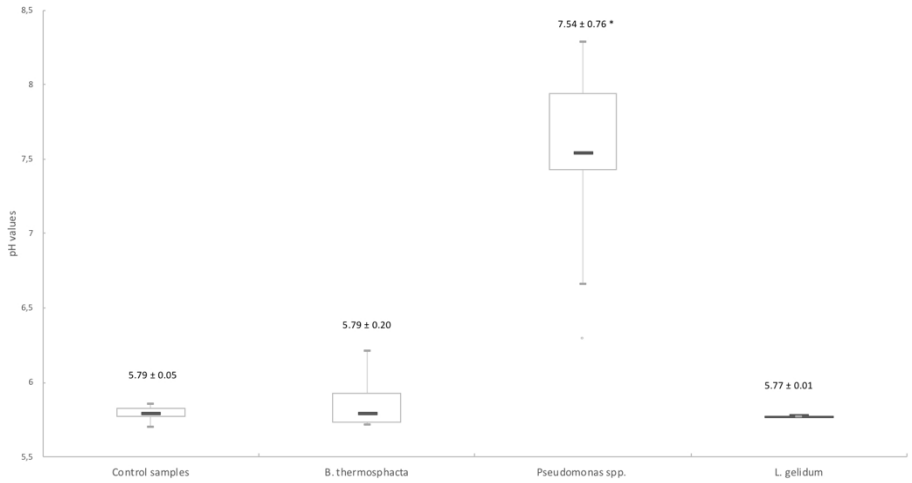

Figure 1: Comparison of pH values for control samples (non-inoculated sterile products) and inoculated products at day 13 for all packaging conditions, * significant statistical difference ($p < 0.05$).

It was also observed a relatively stable composition of MAP packaging in control samples at day 0 (29.6 ± 0.53, n=13) and at day 13 (30.1 ± 1.62, n=12). But it can be observed significant statistical differences for each challenge test, at day 13, according to the temperature condition (Figure 2). The highest values were obtained with *Pseudomonas fragi*, which reached a CO_2 values of 100.0 ± 0.1% at 12°C.

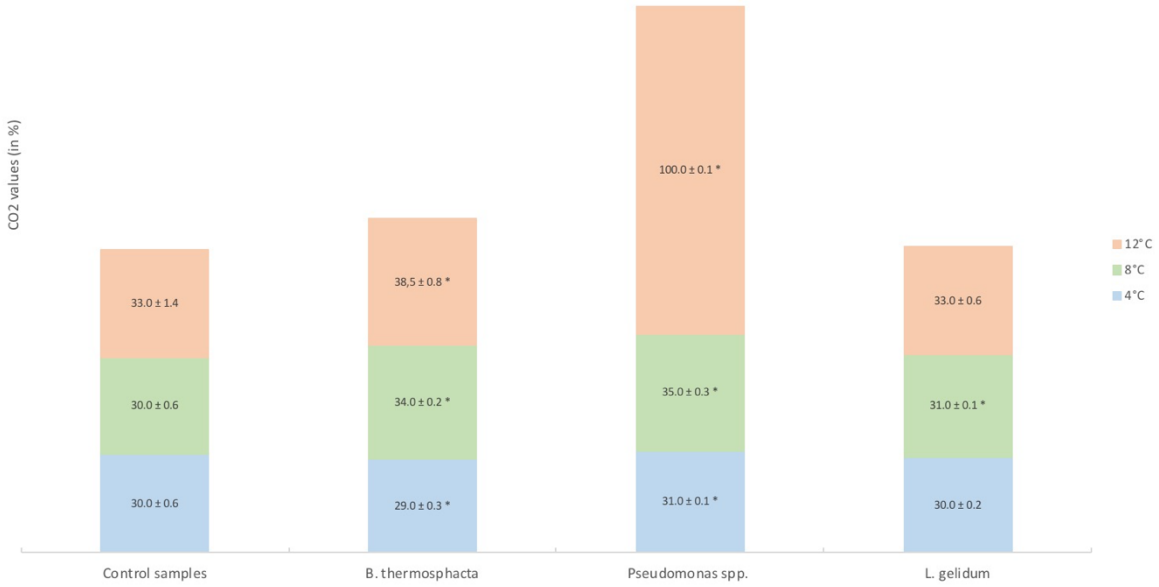

Figure 2: Comparison of CO_2 measurements for control samples (non-inoculated sterile products) and inoculated products at day 13 for modified atmosphere packaging conditions, * significant statistical difference ($p < 0.05$).

3.3 NMR patterns and metabolites

The multivariate analysis (PLS-DA) reveals a clear discrimination between: (i) the non-inoculated products at day 0 and 13 (Figure 3), (ii) the inoculated and non-inoculated samples (Figure

4), (iii) the type of bacterium inoculated on the samples (Figure 5), and (iv) the packaging conditions (Figure 6).

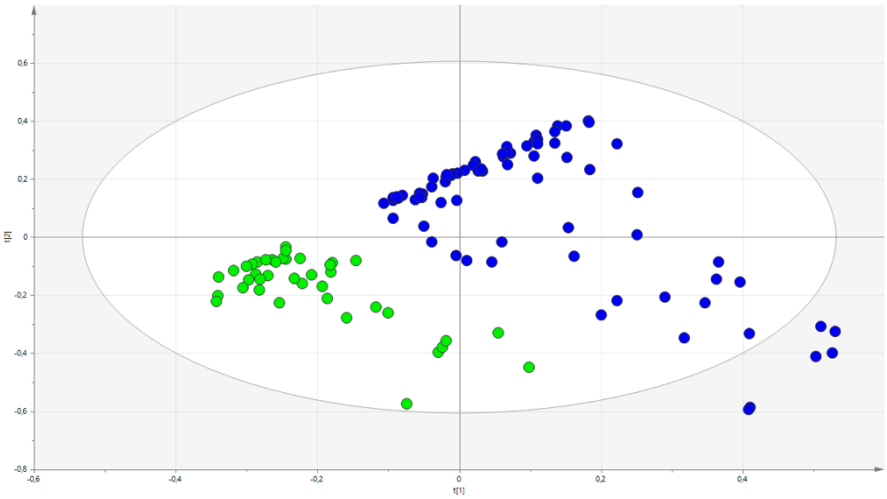

Figure 3: Comparison of metabolomic profiles between non-inoculated samples at day 0 (green circles) and day 13 (blue circles) (R^2=0.925, Q^2=0.903).

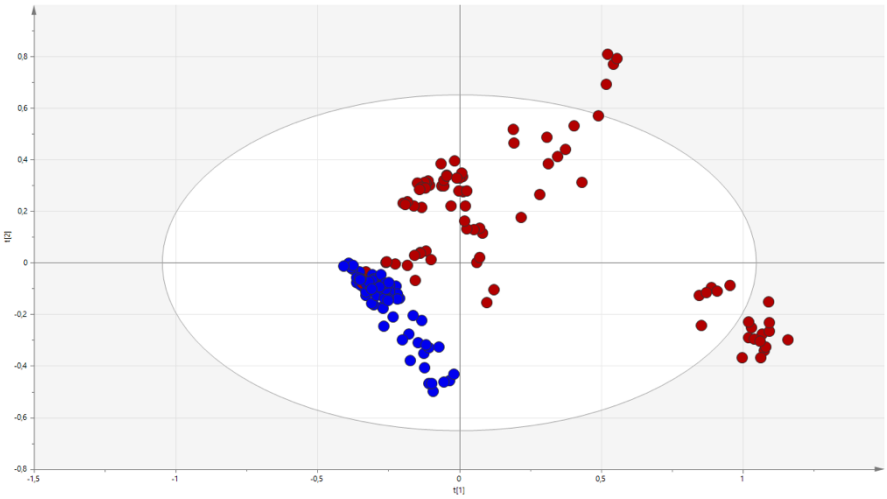

Figure 4: Comparison of metabolomic profiles between non-inoculated (blue circles) and inoculated samples (red circles) at day 13 (R^2=0.858, Q^2=0.838).

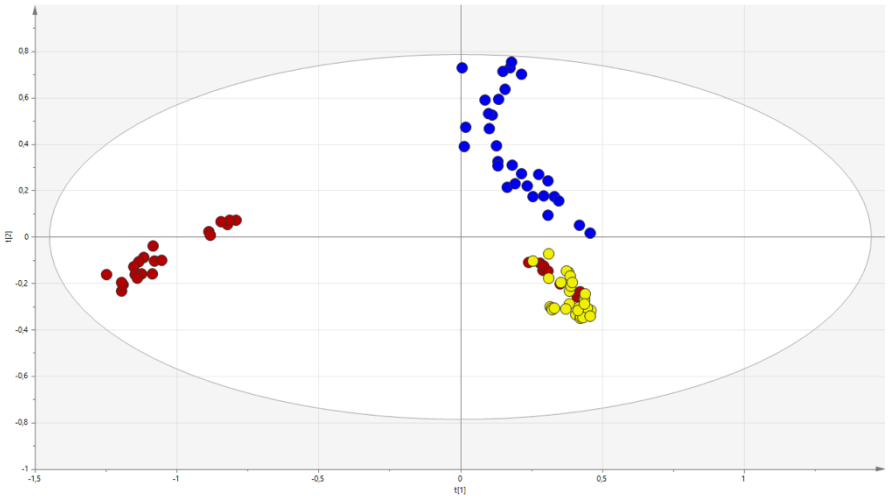

Figure 5: Comparison of metabolomic profiles at day 13 between each inoculated bacterium on samples: *Brochothrix thermosphacta* (blue circles), *Pseudomonas fragi* (red circles) and *Leuconostoc gelidum* (yellow circles) (R^2=0.842, Q^2=0.830).

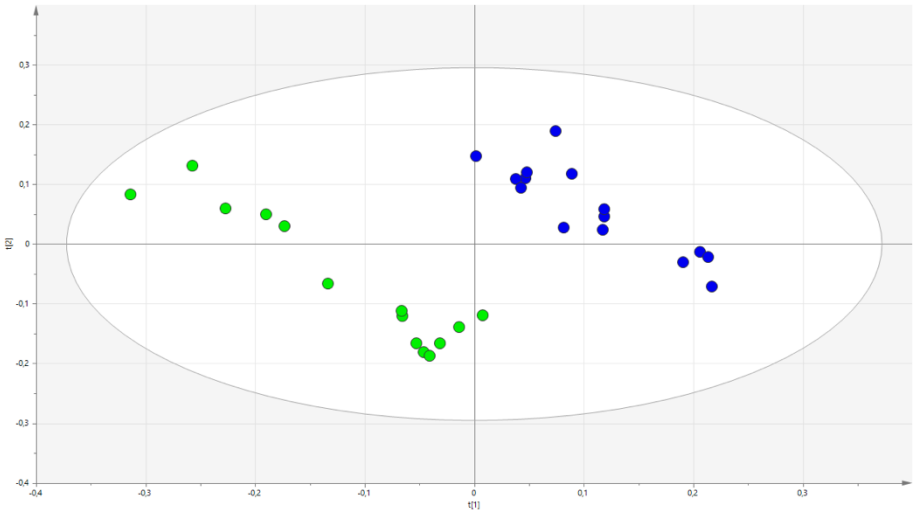

Figure 6: Comparison of metabolomic profiles between each packaging at day 13: modified atmosphere (bleu circles) and food wrap (green circles) (R^2=0.985, Q^2=0.967).

It can be observed that the type of bacterium inoculated had a higher impact on the metabolome than that of the packaging conditions.

Moreover, some metabolites are significantly increased: acetate and acetoin for *B. thermosphacta*, lipoproteins and glutamine for *Pseudomonas fragi*, betaine and lactate for *L. gelidum*. But this part of study needs to be studied more deeply.

4 DISCUSSION

Exploration of the correlations of NMR-based metabolomics results with others microbial parameters suggested their use to provide information on minced pork meat spoilage. Spoilage occurs when the formation of off-flavors, off-odors, discoloration, slime, or any other changes in physical appearance or chemical characteristics make the food unacceptable levels (Ercolini *et al.*, 2011). According to this, the qualitative and quantitative analyses of metabolic compounds present as a

consequence of microbial activity have been considered as a more integrated holistic approach, in which meat quality can be estimated regardless of storage conditions (e.g. temperature, type of packaging) (Argyri *et al.*, 2015). These informations can also be added to other microbial results such as microbiological counts, pH changes and gas composition.

In the food wrap packaging, *Pseudomonas fragi* reached the highest microbiological counts at the end of day 13. And all bacteria reach stationary phase in modified atmosphere packaging. A high growth rate and a more rapidly reached stationary phase are also correlated with the highest storage temperatures. These results are of interest because these bacteria are responsible for unpleasant odours and flavours making the product inedible and are considered as specific spoilage organisms in meat products (Pothakos *et al.*, 2015; Saraoui *et al.*, 2017). *Pseudomonas fragi* also shows the highest values of pH comparatively to the control samples, and the modifications of CO_2 in the MAP packaging is also more important at 12°C for *P. fragi*. The change in pH of products is usually a good index for quality assessment. Indeed, it is commonly related to the accumulation of lactic acid generated in anoxic condition (Aru *et al.*, 2016).

Although many of the spoilage bacteria are proteolytic, they grow initially by utilizing the most readily available carbohydrates and nonprotein nitrogen. Glucose, lactic acid, and certain amino acids, followed by water-soluble proteins, are the precursors of metabolites that are responsible for meat spoilage. Moreover, concentrations of the precursors can influence the rate and extent of spoilage. It is the accumulation of microbial metabolites, such as aldehydes, ketones, esters, alcohols, organic acids, amines, and sulphur compounds, that determines the spoilage of meat (Ercolini *et al.*, 2011). As metabolites may be considered final downstream products of the genome and its interaction with the environment, the determination of metabolites might reveal interesting relationships between food consumption and possible variations in metabolic pathways (Castro-Puyana and Herrero, 2013). The data allow to reveals a clear discrimination between all tested conditions in this study: (i) the non-inoculated products at day 0 and 13, showing the natural evolution of the meat ecosystem according to the storage conditions; (ii) the inoculated and non-inoculated samples; (iii) the type of bacterium inoculated on the samples, reveals a clear discrimination on the metabolite profiles for *B. thermosphacta*, *P. fragi* and *L. gelidum*; and finally (iv) the packaging conditions, between MAP and FW packaging. In the next studies, these patterns will be more deeply studied. Finally, metabolites and their pathways could give important information about the spoilage capacity of inoculated bacteria, and particularly for *Pseudomas fragi* which seems to be of interest in our product.

5 CONCLUSIONS

These results support the use of NMR-based metabolomics as a valuable tool to provide information on minced pork meat spoilage and to follow intrinsically the evolution of the metabolomics pattern linked to a specific bacterium in a complex bacterial ecosystem. The data also suggest that NMR-based metabolomics is an efficient method to distinguish fingerprinting difference between samples, and metabolites can be possible biomarkers of spoilage products.

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REFERENCES

Argyri, A. A., Mallouchos, A., Panagou, E. Z., & Nychas, G. J. E. (2015). The dynamics of the HS/SPME-GC/MS as a tool to assess the spoilage of minced beef stored under different packaging and

temperature conditions. *International Journal of Food Microbiology*, *193*, 51-58. https://doi.org/10.1016/j.ijfoodmicro.2014.09.020.

Aru, V., Pisano, M. B., Savorani, F., Engelsen, S. B., Cosentino, S., & Marincola ; C. F. (2016). Metabolomics analysis of shucked mussels' freshness. *Food Chemistry*, *205*, 58-65. https://doi.org/10.1016/j.foodchem.2016.02.152.

Boffo, E. F., Tavares, L. A., Tobias, A. C. T., Ferreira, M. M. C., & Ferreira, A. G. (2012). Identification of components of Brazilian honeys by 1H NMR and classification of its botanical origin by chemometric methods. *Food Science Technology*, *49*, 55-63. https://doi.org/10.1016/j.lwt.2012.04.024.

Castejon, D., Garcia-Segura, J. M., Escudero, R., Herrara, A., & Cambero, M. I. (2015). Metabolomics of meat exudate: its potential to evaluate beet meat conservation and aging. *Analytica Chimica Acta*, *901*, 1-11. https://doi.org/10.1016/j.aca.2015.08.032.

Castro-Puyana, M., & Herrero, M. (2013). Metabolomics approaches based on mass spectrometry for food safety, quality and traceability. *Trends in Analytical Chemistry*, *52*, 74-87. https://doi.org/10.1016/j.trac.2013.05.016.

Charlton, A. J., Farrington, W. H. H., & Brereton, P. (2002). Application of 1H NMR and multivariate statistics for screening complex mixtures: quality control and authenticity of instant coffee. *Journal of Agricultural and Food Chemistry*, *50*, 3098-3103. https://doi.org/10.1021/jf011539z.

Chen, D. Ye, Y., Chen, J., & Yan, X. (2016). Evaluation of metabolomics profile of crab paste during fermentation. *Food Chemistry*, *192*, 886-892. https://doi.org/10.1016/j.foodchem.2015.07.098.

Consonni, R., & Cagliani, L. R. (2008). Geographical characterization of polyfloral and acacia honeys by nuclear magnetic resonance and chemometrics. *Journal of Agricultural and Food Chemistry*, *56*, 6873-6880. https://doi.org/10.1021/jf801332r.

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. *Applied and Environmental Microbiology*, *77*, 7372-7381. https://doi.org/10.1128/AEM.05521-11.

Jung, Y., Lee, J., Kwon, J., Lee, K. S., Ryu, D. H., & Hwang, G. S. (2010). Discrimination of the geographical origin of beef by 1H NMR-based metabolomics. *Journal of Agricultural and Food Chemistry*, *58*, 10458-10466. https://doi.org/10.1021/jf102194t.

Lipinski, B., Hanson, C., Lomax, J., Kitinoja, L., Waite, R., & Searchinger, T. (2013). Reducing food loss and waste. World Resource Institute (June), 1-40. https://doi.org/10.2499/9780896295827_03.

Mannina, L. Sobolev, A. P., & Viel, S. (2012). Liquid state 1H high field NMR in food analysis. *Progress in Nuclear Magnetic Resonance Spectroscopy*, *66*, 1-39. https://doi.org/10.1016/j.pnmrs.2012.02.001.

Miks-Krajnik, M., Yoon, Y. J., Ukuku, D. O., & Yuk, H. G. (2016). Identification and quantification of volatile chemical spoilage indexes associated with bacterial growth dynamics in aerobacilly stored chicken. *Journal of Food Science*, *81*, 2006-2014. https://doi.org/10.1111/1750-3841.13371.

Piras, C., Marincola, F. C., Savorani, F., Engelsen, S. B., Cosentino, S., Viale, S., & Pisano, M., B. (2013). A NMR metabolomics study of the ripening process of the Fiore Sardo cheese produced with autochothonous adjunct cultures. *Food Chemistry*, *141*, 2137-2147. https://doi.org/10.1016/j.foodchem.2013.04.108.

Pothakos, V., Devlieghere, F., Villani, F., Björkroth, J., & Ercolini, D. (2015). Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Science*, *109*, 66-74. https://doi.org/10.1016/j.meatsci.2015.04.014.

Remenant, B., Jaffres, E., Dousset, X., Pilet, M.-F., & Zagorec, M. (2015). Bacterial spoilers of food: behavior, fitness and functional properties. *Food Microbiology*, *45*, 45-53. https://doi.org/10.1016/j.fm.2014.03.009.

Ritota, M., Casciani, L., Failla, S., & Valentini, M. (2012). HRMAS-NMR spectroscopy and multivariate analysis meat characterization. *Meat Science*, *92*, 754-761. https://doi.org/10.1016/j.meatsci.2012.06.034.

Saraoui, T., Cornet, J., Guillouet, E., Pilet, M.F., Chevalier, F., Joffraud, J.J., & Leroi, F. (2017). Improving simultaneously the quality and safety of cooked and peeled shrimp using a cocktail of

bioprotective lactic acid bacteria. *International Journal of Food Microbiology*, *241*, 69-77. https://doi.org/10.1016/j.ijfoodmicro.2016.09.024.

Yuan, Y., Zhao, Y., Yang, J., Jiang, Y., Lu, F., Jia, Y., & Yang, B. (2017). Metabolomic analyses of banana during postharvest senescence by 1H-high resolution-NMR. *Food Chemistry*, *218*, 406-412. https://doi.org/10.1016/j.foodchem.2016.09.080.

Zanardi, E., Caligiani, A., Palla, L., Mariani, M., Ghidini, S., Di Ciccio, P. A., Palla, G., & Ianieri, A. (2015). Metabolic profiling by 1H NMR of ground beef irradiated at different irradiation doses. *Meat Science*, *103*, 83-89. https://doi.org/10.1016/j.meatsci.2015.01.005.