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Abstract

Sales of fresh duck fatty livers (foies gras) are mainly concentrated during the festive season, creating an imbalance between French consumer demand and product availability. As a result, some operators on the French market are tempted to freeze duck fatty livers in order to sell them fresh, a practice prohibited by French and European regulations. Slaughterers would like to be able to qualify products sold fresh, to ensure that they comply with regulations. To meet this objective, we developed a methodology for detecting fatty livers that have undergone a freezing-thawing process. In our study, 5 methods were tested: oxidation measurement , fingerprinting by MALDI-TOF (Matrix-Assisted Laser Dissociation ionization - Time_Of-Flight) mass spectrometry, near infrared spectroscopy (NIRS), Nuclear Magnetic Resonance (NMR), MRI and conductivity. In the first phase of the project, MALDI-TOF fingerprints were able to classify livers from the same batch, calibrated by weight, with internal validation and recognition percentages ranging from 93 % to 100 %. Similarly, the NIRS method showed that it was possible to develop calibrations with model determination coefficients (R^2c) of 0.82, and cross-validation coefficients (R^2cv) of 0.80. In the second phase of the project, variability was incorporated into the origin of the livers, in order to provide a model specific to freezing/thawing and usable on all-origin livers. The MALDI-TOF and NIRS methods were able to recognize frozen fatty livers, but with classification errors and too much variability to validate the first-phase model. A validation phase of these two methods on a larger pool of fatty livers, from different technical itineraries seems necessary to have robust and reliable prediction equations. As for NMR and MRI measurements, despite encouraging results in the first phase, they could not be repeated on fatty livers of various origins (different slaughterhouses) due to poor control of the cold chain between sampling and analysis.

Keywords: duck, fatty livers, MALDI TOF, NIRS, NMR, MRI

1. Introduction

Sales of fresh duck foie gras are mainly concentrated around the end-of-year festive period, which leads to an imbalance between demand from French consumers and availability of the product. Some players on the French market are therefore tempted to freeze duck foie gras in order to sell it fresh during the festive season, which is a prohibited practice (*Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 on the common organisation of agricultural markets and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007*, 2013). Poultry meat and poultry meat preparations can only be marketed fresh, frozen or deep-frozen. The marketing of defrosted poultry livers is therefore not authorised in France or Europe. It is currently impossible to identify these thawed foies gras, whereas being able to qualify products sold fresh on the French market would make it possible to ensure that they comply with the regulations, especially over the festive period, in order to avoid unfair competition by regulating the market. What's

more, consumers cannot detect thawed duck foie gras with the naked eye, and to protect them we need to have a method of certifying that it is indeed a fresh product.

The development of reliable and objective analytical tools, capable of discriminating between products that have undergone a freezing/thawing stage before being marketed chilled, could help to avoid fraudulent practices and contribute to ensuring greater fairness in commercial transactions. Five methods were tested during the project: measurement of product oxidation, fingerprinting by MALDI-TOF mass spectrometry, near infrared spectroscopy (NIRS), nuclear magnetic resonance (NMR), magnetic resonance imaging (MRI) and conductivity.

Protein spectral fingerprinting using MALDI-TOF mass spectrometry has, in recent years, become the reference method for identifying bacteria in hospitals (Nomura, 2015). The spectrum of proteins extracted from a bacterium (or spectral fingerprint) is compared with a database and a statistical score is given by the system to validate or not the identification. Very recently, studies have also shown its relevance for controlling the shelf life of trout by creating a prediction tool (Ulrich *et al.*, 2017). Shelf-life discrimination (0, 3, 7, 9 and 11 days) was achieved with a classification score above 90%. In addition, this spectral method was used to create a database of 54 fish species for monitoring adulteration in the fish farming industry (Stahl and Schröder, 2017).

Near infrared spectroscopy (NIRS) is a rapid, non-invasive technique for predicting the chemical composition of food products. NIRS has been successfully used for many years on the meat matrix to predict chemical composition for several species: intramuscular lipid content is predicted with high-fit models for pork (R^2 = 0.84, Liao *et al*, 2010), lamb (R^2 = 0.84, Andrés *et al.*, 2007), beef (R^2 = 0.99, De Marchi *et al.*, 2007), duck (R^2 = 0.84, Bastianelli *et al.*, 2009) and chicken (R^2 = 0.83, Chartrin P., 2010). For matrices that are richer in lipids and whose composition is closer to that of fresh foie gras, such as fat/lean mixtures or sausages, NIRS prediction models also show a high level of accuracy with lipid content (R^2 = 0.83 and 0.99, Gaitán-Jurado *et al.*, 2008; Tøgersen *et al.*, 1999). There are fewer data available on the specific prediction of the chemical composition of fresh foie gras, but they show promising results, particularly for lipid content (R^2c = 0.81 to 0.89; Marie-Etancelin *et al.*, 2011; Molette *et al.*, 2001). NIRS was also able to identify 95% to 98% of beef steaks that had been frozen and then thawed (Thyholt and Isaksson, 1997). The same technique applied to fish enabled Uddin and Okazaki (2004) to discriminate without error between fresh mackerel and mackerel that had been frozen and then thawed.

NMR and MRI can be used to highlight the structure of the matrices studied and the state of the water within these matrices, particularly food matrices. The NMR method is based on the detection of protons, the hydrogen nuclei that make up molecules. In food science, water and lipids are the molecules most studied using these techniques, due to their abundance in foods and their easily measurable molecular mobility via the relaxation times T1 and T2, intrinsic parameters of the technique. The use of NMR relaxometry on animal flesh makes it possible to predict water retention capacity (Bertram *et al.*, 2002; Gudjonsdottir *et al.*, 2010; Lambelet *et al.*, 1995) and thus to quantify the exudate produced after thawing the product. It has also been shown that the type of cooling used on pork meat or fish flesh leads to a difference in T2 relaxation times for water (Bertram *et al.*, 2002; Gudjonsdottir *et al.*, 2010). MRI, based on the same physical principles as NMR but with a spatial resolution of protons, has been used to study the effect of freezing/thawing mainly on fruit and vegetables, meat and fish. A study of beef, pork and lamb concluded that the natural variability of the products made it difficult to identify fresh products from frozen-thawed products (Evans *et al.*, 1998). The indicators used were T1, T2 and magnetisation transfer rate (MTR). The same indicators were used on cod and mackerel (Nott *et al.*, 1999, 2.35T MRI). Signal variations on T1- and T2-weighted images were studied on Turbot and showed a signal evolution in the images after several freeze/thaw cycles (Li *et al.*, 2018). Thus on the basis of the cited publications, the combination of NMR and MRI techniques, known for their non-invasive and non-destructive character, seem appropriate to study the phenomena related to freezing/thawing of products such as raw foie gras.

2. Materials and methods

2.1 Preparing the livers

The study was divided into 2 successive phases.

In the first phase of the study, measurements and sampling were carried out on the same livers (n = 100), in order to avoid any variability and to ensure that the results obtained were directly related to the freezing-thawing process. Each of the livers was cut in half (sagittal section) and the two parts were alternately assigned to a measurement on fresh liver on the day of slaughter (D0) and then after 2 weeks of storage at 5°C (D14), or to a measurement on livers frozen for 6 months and thawed (M6), then to an additional measurement after 2 weeks of storage at 5°C (M6+D14).

During the second phase of the study, several modalities were integrated in order to increase the sources of non-specific variability of the freezing/thawing process and to get closer to the reality in the field (Table 1):

- **Individual**: the freeze-thaw treatment is applied to a population different from the control.
- **Weight**: 3 ranges of livers (<500g, 500-600g, >600g)
- **Storage temperature for fresh liver**: 3 temperatures (4°C, 0°C and 8°C)
- **Fresh** shelf **life**: 3 conditions (0 days, 7 days, 14 days)
- **Freezing time**: 2 times (3 months and 6 months)
- **Slaughterhouse of origin**: 3 slaughterhouses

Table 1 Modalities tested during the second phase of the project, incorporating variability in order to get closer to the reality on the ground.

Treatment	Shelf life (in days)	Preservation (temperature in °C)	Weight range	Slaughterhouse 1	Slaughterhouse 2	Slaughterhouse 3
Fresh	-	-	< 500g	5	5	5
			500 - 600g	5	5	5
			> 600g	5	5	5
Fresh	7	0	< 500g			5
			500 - 600g			5
			> 600g			5
Fresh	7	4	< 500g		5	5
			500 - 600g		5	5
			> 600g		5	5
Fresh	7	8	< 500g		5	
			500 - 600g		5	
			> 600g		5	
Fresh	14	4	< 500g	5	5	5
			500 - 600g	5	5	5
			> 600g	5	5	5
Frozen in January (M6)	2	4	< 500g	5		6
			500 - 600g	5		7
			> 600g	5		7
Frozen in January (M6)	7	4	< 500g	6		
			500 - 600g	7		
			> 600g	7		
Frozen in January (M6)	14	4	< 500g			6
			500 - 600g			7
			> 600g			7

Frozen in March (M3)	2	4	< 500g	5	6
			500 - 600g	5	7
			> 600g	5	7
Frozen in March (M3)	7	4	< 500g	6	
			500 - 600g	7	
			> 600g	7	
Frozen in March (M3)	14	4	< 500g		6
			500 - 600g		7
			> 600g		7

2.2 MALDI-TOF

Sample preparation prior to MALDI-TOF analysis was optimised using the method published by Théron *et al* (2020). The final step involved statistical chemometric analyses of MALDI-TOF protein spectral data using ClinProTools software. Firstly, the parameters for determining the peak list were optimised (signal to noise ratio of 3, Savitzy-Golay spectral smoothing algorithm). In order to classify the livers, a prediction model was built based on the neural network algorithm. To achieve this, a cross-validation approach was used: 80% of the data is used to build the model and the remaining 20% to validate it, i.e. classified using the model. These data are selected at random by the software and the operation is repeated 10 times to be as complete and representative as possible. The neural network proved to be the most relevant for discriminating between foie gras according to the method of preservation/freezing. The model created was validated internally by cross-validation and externally with a new set of data.

2.3 Near infrared spectrometry

The equipment used consisted of a Labspec4 ASDI spectrometer (350-2500 nm) equipped with its contact probe (2 cm diameter window). The spectral resolution is 3 nm in the 350-1000 nm range and 10 nm in the 1000-2500 nm range. The integration time was set at 10 msec and each spectrum is the average of 10 repetitions, giving a total measurement time of one second per sample. The spectra are expressed in reflection index. Calibrations were carried out using Matlab software and the Eigenvector toolbox, and the PLS-DA (Partial Least Squares Discriminant Analysis) procedure. The prediction models were determined by monitoring the cross-validation error as a function of the number of PLS factors included in the model (20 random draws). Model performance was then assessed by the prediction error obtained on an external validation dataset. To calculate the R^2, the numerical value of the class (0 or 1) was taken into account, which nevertheless provides a point of comparison between the level of fit of several models. The most important performance criterion remains the percentage of livers that are well classified according to the probability of belonging to a class provided by the model.

2.4 NMR and MRI spectroscopic and imaging methods

Unlike the other methods, the analyses were carried out in the laboratory due to the non-mobility of the measuring equipment, particularly the MRI. The foie gras were delivered by refrigerated lorry at around 4°C and kept at this temperature in a cold room. D0 was the day following delivery, and D14, two weeks after D0. For phase 2, fresh and frozen livers were collected from the abattoir and sent to the laboratory at 4°C, where they were received the following day.

NMR measurements were carried out on a 20 MHz spectrometer (minispec, Bruker) using cylindrical samples approximately 8 mm in diameter and 1 cm high placed in dedicated glass tubes. Three samples were taken per liver. T2 relaxation times were measured at different temperatures (-10°C, 4°C, 10°C, 20°C, 60°C, 70°C, 80°C, 90°C) via the FID-CPMG (Free Induction Decay-Carl Pucell Meiboom & Gil) sequence (Meiboom and Gill, 1958), and T1 relaxation times via the FSR (Fast Saturation Recovery) sequence (Fukushima and Roeder, 2018) at 4°C. The T1 measurements were carried out in order to

optimise the T2 measurements (FID-CPMG sequence with a recycling time of 4 s). At 4 °C, these used an echo time of 0.1 ms and the accumulation of 2,600 echoes. To extract T2 relaxation times from the raw data, each signal was modelled according to the following equation 1:

$$I(t) = \sum_i A_i e^{-t/T_{2i}} \qquad \text{Equation 1}$$

Where A_i represents the amplitude of the ith component characterised by a relaxation time T_{2i} .

An analysis of variance was performed on all T2 measurements using Statgraphics software, using the ANOVA F-test to determine significant differences between the means of the fatty liver groups.

MRI was used in this project with three distinct protocols. Two protocols were aimed at estimating parameter mappings, namely T2 relaxation time and lipid content. A single two-dimensional plane was produced, with a spatial resolution of 1.5 x 1.5 mm^2 and a slice thickness of 4 mm, for both protocols. The aim of the third protocol was to observe any changes in the macroscopic structure, using 3D acquisition with a spatial resolution of 1 x 1 x 0.8 mm^3 . MRI images were acquired on a Siemens Avanto 1.5T scanner, with the option of analysing livers at different temperatures. Measurements were taken at 4°C and 25°C. To minimise spatial variations in image intensity, the livers were analysed one by one, positioned in the same location. The 3D acquisitions were carried out in pairs because of their longer duration. The measurement of lipid content is based on the frequency difference between the water and lipid signals, which makes it possible to separate these two signals, calculate a ratio and analyse them separately (Hu *et al.*, 2012).

3. Results and discussion

3.1 MALDI-TOF

The 600 individual protein spectra acquired as part of phase 1 are shown in Figure 1.

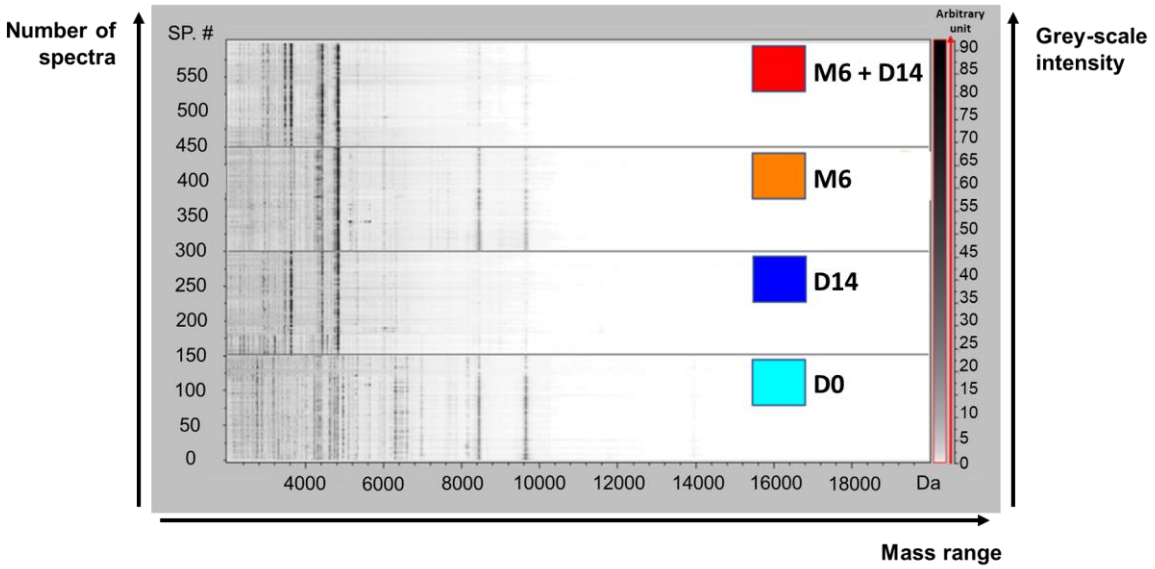

Figure 1 Visualisation of MALDI-TOF spectra obtained on the livers analysed during phase 1.

The gel display shows inter- and intra-individual variability. This representation shows that certain differences are present between the 4 conditions, with the presence of peaks (represented by the vertical bars in Figure 1) only in the conditions without conservation at 4°C, for example. Statistics can be used to determine whether these differences are significant.

The results obtained have been evaluated on the basis of the recognition capacity provided by the software. This was an assessment of the model's ability to classify new data on the basis of the input dataset.

The results are shown by class in Figure 2.

In the case of foie gras fingerprints, the results were positive and homogeneous between classes, with internal validation and recognition capacity percentages ranging from 93% to 100%. This result for the ability to predict the freezing and preservation of foie gras is based on the intensity of 24 peaks over the entire mass range analysed. The intensities of these 24 peaks form the equation for predicting belonging to the 4 classes.

During the second phase of the project, the emphasis was placed on taking into account the variability of conditions potentially encountered in the field.For this second phase, it was decided to combine the different conditions, grouping livers regardless of their weight range and regardless of the slaughterhouse from which they were taken, since this should not be the predominant information in a good field-usable classification method..

This approach is simpler to understand as it only covers the 4 classes described in phase 1, i.e. D0, D14, M6 and M6+D14. The classification results by grouping the conditions from phase 2 are shown in Figure 3.

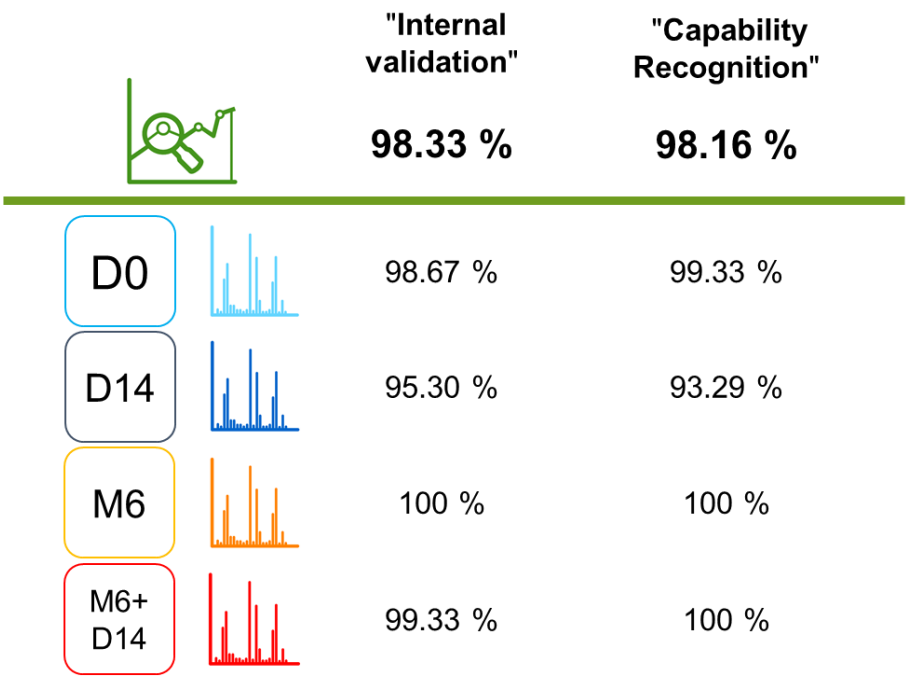

Figure 2 Classification of the livers during phase 1 by MALDI TOF fingerprinting

	D0	D14	M6	M6+ D14
Fresh foie gras	1	36	97	4
Preserved fresh foie gras	60	58	197	1
Frozen/thawed foie gras	12	33	165	1
Frozen/thawed preserved foie gras	55	126	33	27

Figure 3 Classification of the livers during phase 2

The diagonal represented by the boxes corresponds to the expected result. Classification according to the 4 classes confirms the detailed results for each condition: frozen foie gras is relatively well recognised by the model. Nevertheless, there are too many misclassifications, and too much variability, to be able to validate the model created with the spectral protein fingerprint data for foie gras from phase 1.

It appears that the model is " over-learned ": it is very specific to the conditions of phase 1, too highly specialized to be used to classify spectral fingerprints acquired during further experiments. When confronted with more realistic data, it loses classification power.

3.2 Near infrared spectrometry

During phase 1 of the project, PLS-DA calibrations (discriminant analysis) based on changes in spectral signatures in the visible and near infrared, revealed good classification performance between fresh half-livers (D0 and D14) and frozen/thawed half-livers (M6 and M6 + D14): coefficients of determination were good in calibration (R^2c = 0.82) and stable in cross-validation (R^2cv = 0.80), while the cross-validation error was 2%.

However, calibration tests were carried out on sub-populations in order to assess the robustness/specificity of the calibrations: in particular, a model was developed for batches measured at D14 and M6 + D14, then an external validation of this model was carried out on batches measured at D0 and D14 and M6 (frozen/thawed). The results in this case were disappointing, with a very sharp drop in the coefficient of determination in external validation (R^2p = 0.41) and a very high error (43%).

These two calibration trials have therefore shown us that it is firstly possible to develop calibrations specific to the freezing/thawing treatment of liver. However, we also conclude that it will nevertheless be essential to incorporate any kind of variability likely to modify the spectral signature in future calibrations in order to have a robust model, specific to freezing/thawing and usable on all types of livers.

During phase 2 of the project, variability in the origin of the livers was incorporated (Table 1). An initial validation analysis was carried out by testing the calibration models from phase 1 on the new population of foie gras. This external validation confirmed the robustness problems that had been highlighted previously: the coefficient of determination of the external validation was very low (R^2p = 0.09) and the classification error was high (34% of livers misclassified). These results prompted us to consider this new population of livers as a database designed specifically for the development of robust calibrations for the detection of frozen/thawed livers on batches of 'any' livers.

In order to meet both the need to develop new, robust calibrations and the external validation model performance testing objectives of phase 2, the population of 300 livers was divided into 2 (Figure 4):

- Calibration/cross-validation sub-population: slaughterhouses 2 + 3 (n = 200)
- External validation sub-population: slaughterhouse1 (n = 106)

External validation / n=106 **Calibration / n=200**

	Slaughterhouse 1		Slaughterhouse 2		Slaughterhouse 3	
Shelf life	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
0 day (T0)	15	20	15	20	15	30
7 days (T7)	30	-	30	-	-	40
14 days (T14)	21	20	15	20	15	-
Total	66	40	60	40	30	70

Figure 4 Distribution of livers for phase 2 (number of livers for each modality)

The results of the calibrations coupled with the external validations revealed models with strong fits: R^2c from 0.60 to 0.96 (calibration), R^2cv from 0.59 to 0.91 (cross-validation) and R^2p from 0.67 to 0.86 (Table 2). The models were therefore generally robust, regardless of the type of spectrum pre-processing used. The simplest models were already accurate, with 13% error for the model applying a simple baseline correction and slight signal smoothing (order 4). However, this model showed a very unbalanced error towards false negatives, which greatly reduced the quality of liver detection. However, the use of the GLSW (General Least Square Weighting) procedure, which consists of an *a priori* selection filter for spectral zones related to the magnitude to be predicted, greatly improved the accuracy of the classification models: only 2% classification error with a fairly balanced false positive/false negative distribution (37%/63%).

Table 2 Summary of calibration results and external validation obtained on the distribution of data from phase 2

Calibrations 2020 Slaughterhouses 2+3 (n=200)				External validation 2020 Slaughterhouse 1 (n=106)		
Pre-treatment	Nb of PLS factors	R^2c	R^2cv	R^2p	% error	% false positive /% false negative
RAW	5	0,81	0,79	0,73	25	0 / 65
AWLS2+SMOOTH4	2	0,60	0,59	0,67	13	0 / 35
AWLS2+SMOOTH4+AUTO	6	0,81	0,77	0,83	31	0 / 83
AWLS2+SMOOTH4+SNV	2	0,62	0,60	0,67	13	0 / 35
AWLS2+SMOOTH4+MSC	5	0,82	0,80	0,86	23	0 / 63
AWLS2+SMOOTH4+GLS (α=0,02)	2	0,91	0,86	0,85	9	0 / 25
AWLS2+SMOOTH4+GLS (α=0,005)	2	0,96	0,91	0,72	2	1,5 / 2,5

Best model PLS-da

3.3 NMR and MRI spectroscopic and imaging methods

3.3.1 NMR

The first phase of the project determined the T2 relaxation parameters of the main constituents of foie gras, water and fat. Their evolution as a function of temperature was studied in order to verify certain physical laws (Curie's and Arrhenius' laws) influencing the relaxation parameters and the intensity of the NMR signal. Figure 5 shows the T2 distributions of samples of foie gras at 4°C and 80°C.

Increasing the temperature (between 4°C and 80°C in this case) leads to a clear disappearance of the first component around 20 µs and an increase in the relaxation times (3), (4) and (5).

By analysing the evolution in relaxation time and mass intensity (not shown here) of each peak in the distribution and comparing it with that of pure water and fat extracted from foie gras, we were able to assign each T2 component (peak) to families of protons.

At 4°C on fresh foie gras (D0), 6 T2 relaxation times were measured:

- The T2(1) around 13 µs would correspond to mainly fats crystallised, with a small fraction of non-exchangeable macromolecule protons.
- The T2(2) and T2(2)' around 550 µs and 2.5 ms, respectively, would correspond to exchangeable protons of macromolecules interacting with water.
- T3(3), T2(4) and T2(5) around 3.5 ms, 15 ms and 50 ms, respectively, would correspond to water/fat mixtures.

The same relaxation times with similar intensities were found for fresh foie gras stored for 14 days at 4°C.

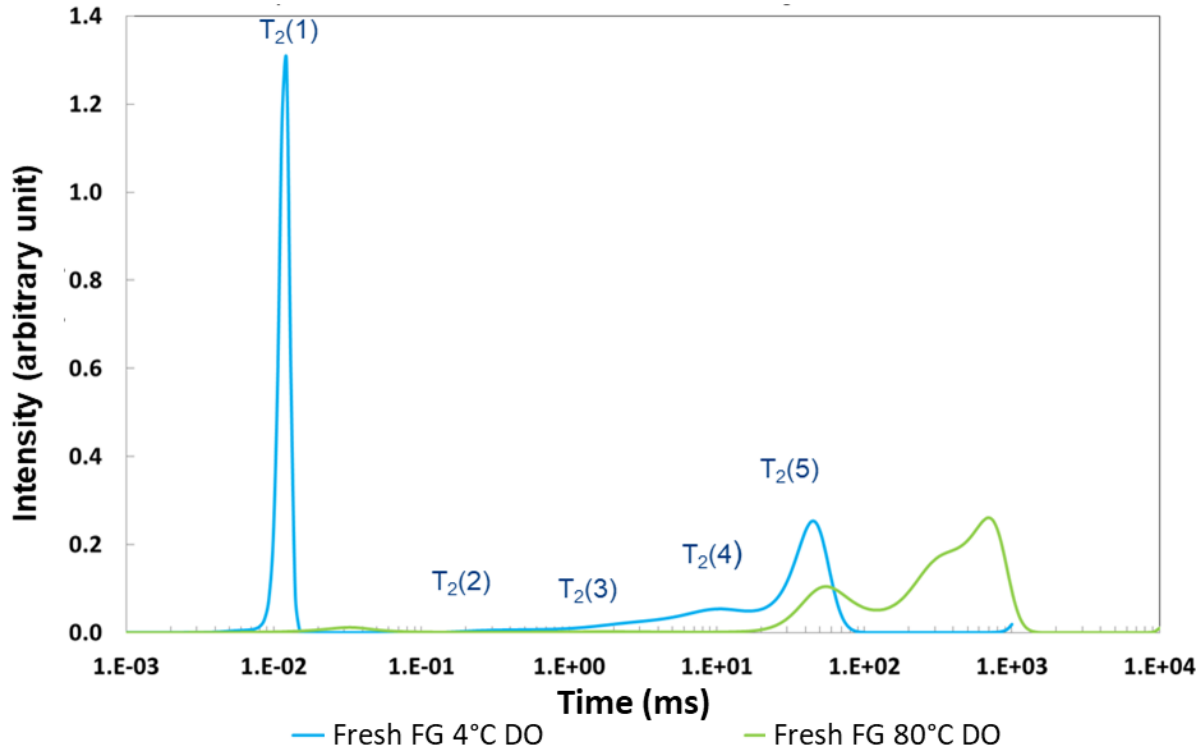

Figure 5 T2 distributions at 4 and 80°C for fresh foie gras samples (d0)

In phase 2, the task was to carry out NMR measurements on 4 types of product: foies gras (FG) fresh on D0, fresh foies gras stored at 4°C for 14 days (D14) and foies gras frozen/thawed for 6 months on D0 and then stored at 4°C for 14 days (D14). NMR measurements were performed on 12 foies gras, taking 3

samples per liver. Following the measurements at 4°C, each sample underwent the thermal processing described in the Materials and Methods section. After mathematical processing of the NMR data to extract the T2 values and their intensity, a statistical analysis was carried out on these different variables to check whether the group averages (FG fresh D0, FG fresh D14, Frozen D, Frozen D14) differed significantly from each other. The statistical tests did not allow a significant distinction to be made between fresh and frozen FG on D0. Indeed, the ANOVA tests indicated fairly low Fisher test values (LSD*, Least* Significant *Difference*) (< 60) between the means of T2 relaxation times or their intensity. Only the analyses carried out by increasing the measurement temperature, and in particular to 40°C, showed significant differences in T2(3) between fresh and frozen/thawed FG. Remember that the T2(3) component is attributed to a mixture of water and fat. It is possible that freezing the FGs impacts the crystallised fat fraction so that thawing and heating to 40°C impacts this proton pool characterised by a specific T2(3). ANOVA of the mean T2(3) values measured on D0 confirmed these significant differences (with a 95% probability) with fairly high F-tests (> 1500), as shown in figure 6. These differences were much less significant after storage at 4°C for 14 days (D14) (F < 50).

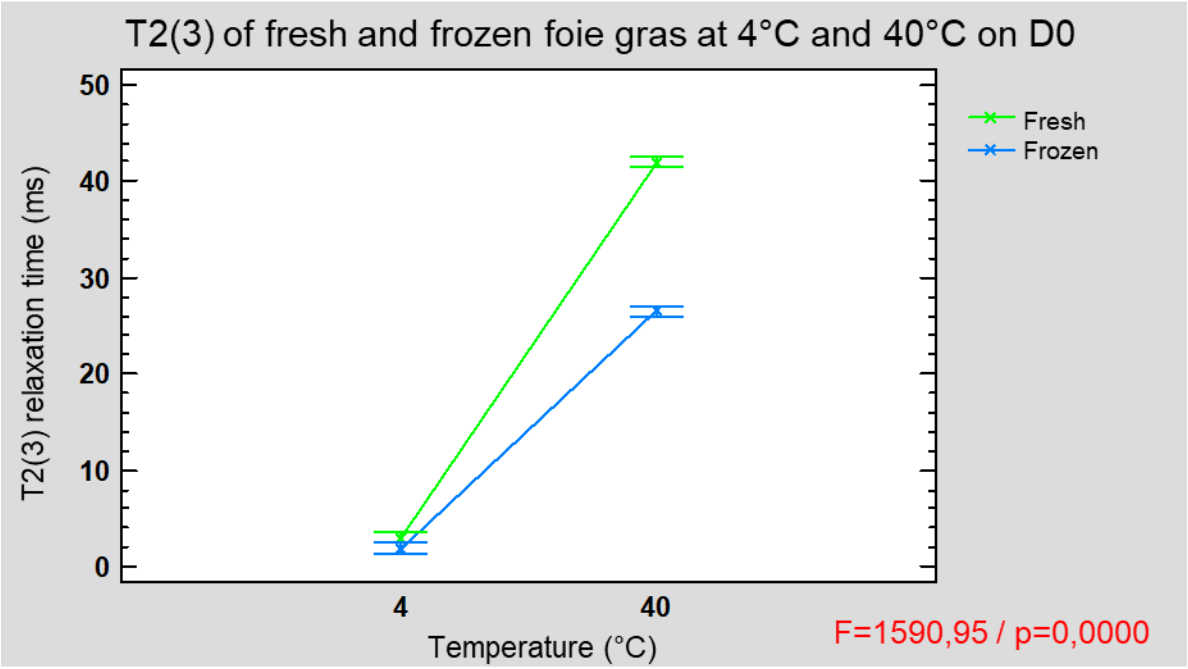

Figure 6 ANOVA tests on variations in the T2(3) component of fresh (F) and frozen/thawed (S) FG samples at D0. In red, the F factor and its probability

By including variability in the origin of the livers in our analyses (Figure 4), the NMR measurements showed that only the fatty livers frozen on D0 differed from the other 3 classes. However, the variability of the samples was such that this distinction did not seem sufficient to us.

Frozen foies gras at D14 merged with fresh foies gras, which had not been observed in the first part of the project. The significant differences observed during measurements at 40°C in phase 2 were not repeated when foies gras from different abattoirs were analysed. These differences were attributed to the different thermal paths undergone by the foie gras between sampling and analysis.

3.3.2 MRI

With regard to the results of the first phase, the visual analysis of the images acquired in 3D did not allow us to detect any differences between the fresh and frozen/thawed livers. In particular, we did not observe any cracks in the frozen/thawed livers that might have appeared during the freezing process. These observations tend to prove that the macroscopic structure of the livers is not sufficiently altered to be observed on a millimetre scale.

Also for the first phase, Figure 7 shows the lipid levels and the mean water signal and lipid signal for fresh livers at D0 and D14 and frozen livers at D0 and D14, as well as their mean T2 and associated amplitude.

We observed a variation in lipid levels measured by MRI, with a decrease between D0 and D14 for fresh livers and a lower value for frozen/thawed livers at D0 and D14. This decrease is linked to the decrease in the lipid signal, as the water signal does not vary according to the days or the thermal history. The decrease in the lipid signal is very probably linked to the increase in the percentage of fat in the crystallised state, which does not give a signal on MRI. There is a crystallisation phenomenon in fresh livers between D0 and D14 and, when the temperature of the frozen livers is raised, more crystallised fat remains than in fresh livers. This suggests that it is not only the temperature but also the thermal history of the livers that has an impact on the rate of crystallisation and therefore on the lipid content measured by MRI. This hypothesis was tested by raising the temperature of 3 frozen livers to 20°C (breaking the cold chain) and then lowering it again to 4°C. It was found that after 6 hours at room temperature, the lipid level measured at 4°C reached a value typical of fresh foie gras. The difference observed is therefore very probably due to a different state of the fat which, in the case of frozen livers, has not decrystallised to the same extent as the lipids in fresh foie gras. This phenomenon is probably also at the origin of the variation in amplitude A0 (Figure 7) since, with this protocol too, crystallised fat does not give a signal. However, there was no significant variation in the mean value of T2.

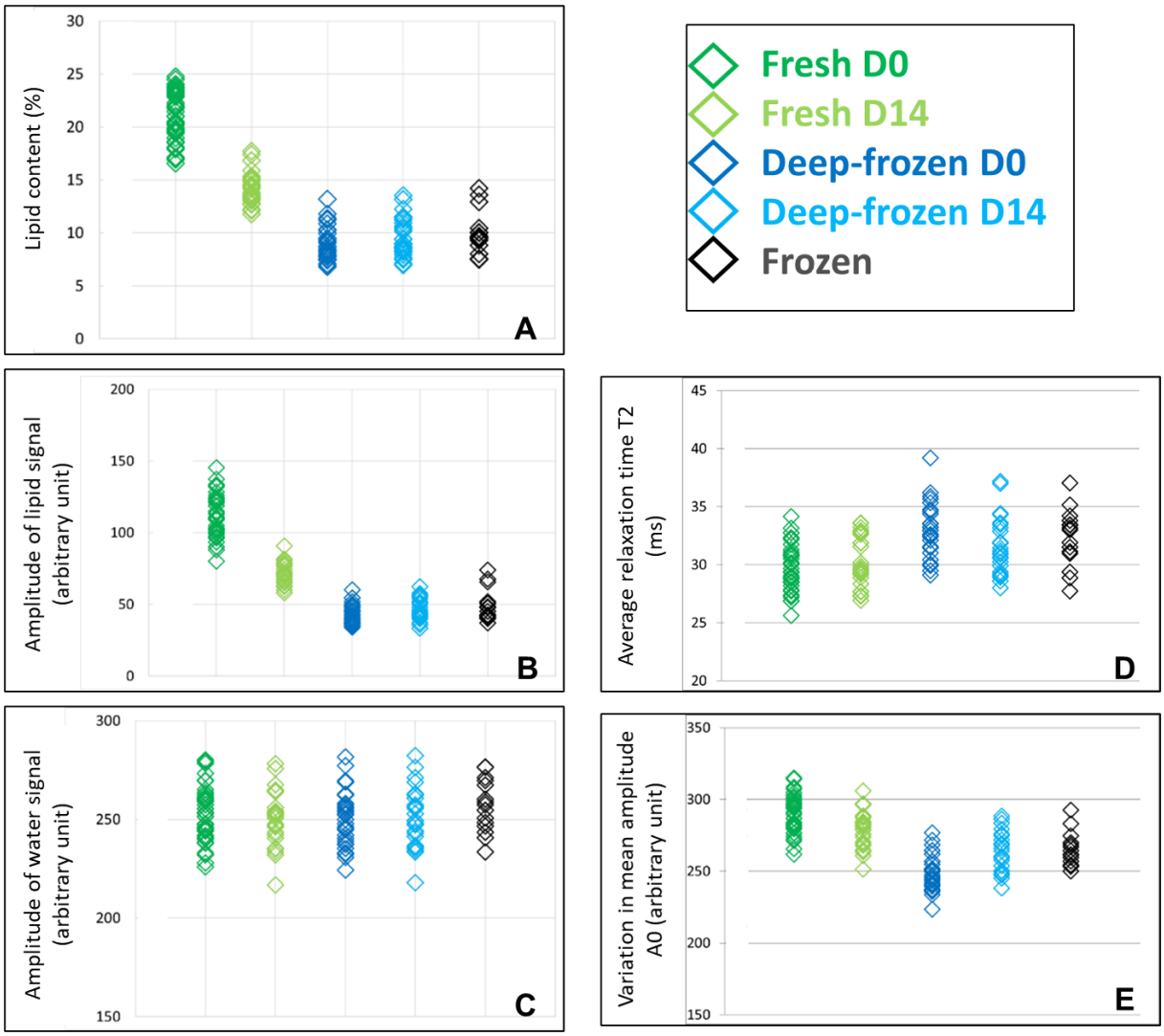

Figure 7 Lipid content (A), mean lipid signal (B) and water signal (C) as well as mean T2 relaxation time (D) and variation in mean amplitude (E) on preserved and unpreserved fresh and frozen fatty livers.

For the second phase, breaks in the cold chain were added to take account of this eventuality. There was also a drop in lipid levels measured for frozen livers on D0, although less marked than that observed in the first phase, which may be explained by greater variability in the origin of the livers. In addition, a 1-hour break in the cold chain was enough to erase the differences between the fresh and frozen livers.

4. Conclusion

Detecting frozen foie gras sold fresh is an important issue for the industry, and developing a reliable and robust detection method for use by the fraud department would help prevent fraudulent practices and ensure fairer commercial transactions. In fact, this type of practice - even if limited in volume - distorts the balance between supply and demand: by creating artificial availability on the fresh foie gras market at the end of the year, it weighs on the industry with a loss of earnings estimated *at a minimum of* 1.2 million euros. MALDI-TOF and NIRS methods could be used to meet this demand. In fact, the accuracy levels achieved by these two methods in the context of a genuine external validation (different site, dates and population) offer very good prospects for the development of an application for the detection of frozen/thawed livers. A combined statistical analysis of the 2 methods could also be considered to improve robustness and reliability. However, the challenge now remains to validate this level of performance on a larger, more representative sample.

Low-field NMR, like MRI, is very sensitive to the rate of fat crystallisation, which depends on the thermal history of the livers. The measurements carried out on this project have shown that, without control of the thermal pathways undergone by foie gras throughout the chain of preservation (in the abattoir, during transport, on shop shelves, in the analysis lab using a strict defrosting protocol), NMR and MRI cannot distinguish between a fresh liver and a frozen/thawed liver.

Ethics

The authors declare that the experiments were carried out in compliance with the applicable national regulations.

Declaration on the availability of data and models

The data supporting the results presented in this article are available on request from the author of the article.

Declaration on Generative Artificial Intelligence and Artificial Intelligence Assisted Technologies in the Drafting Process.

The authors used artificial intelligence in the translation process from French to English.

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Authors' contributions

All the authors contributed to the writing of this article

Declaration of interest

The authors declare that they do not work for, advise, own shares in, or receive funds from any organisation that could benefit from this article, and declare no affiliation other than those listed at the beginning of the article.

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