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RESEARCH ARTICLE

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Foodomics and storage monitoring of three meat cuts by ^{1}H NMR

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Abstract
The growth of NMR foodomics is described in the context of a study of beef storage. Thirty samples of three meat cuts (chuck, sirloin, and tenderloin) were analyzed using ^{1}H NMR spectroscopy to determine the influence of storage period and temperature. ^{1}H showed signals belonging to metabolites namely: acetate, adenosine, adenine, ADP, alanine, betaine, creatine, creatinine, carnosine, fumarate, glycerol, glycine, glutamine, isoleucine, lactate, leucine, methionine, and valine. The score plots (PCA) separated the samples of different storage time, reflecting possible meat degradation. Samples of no storage time (time zero) were grouped in the PC1 and PC2 negatives axis. The score plots suggest that the temperature has a huge influence on the degradation extent and possible influences the growth of the microbial populations.

KEYWORDS
^{1}H NMR, chemometrics, foodomics, meat cut storage, metabolites

1 | INTRODUCTION

Premature spoilage of Brazilian vacuum-packaged chilled beef is one of the main challenges that the industry is facing today, affecting the commercialization and consumption of beef in the country and abroad. Considering the role of Brazil in the world scenario of beef production, a thorough study of the conditions impacting meat quality was undertaken.

Fresh meat is recognized as a highly perishable food, and its vacuum packaging and storage at low temperatures are strategies commonly used to delay microbial spoilage, thus maintaining its quality.[1-4]

Metabolomics can monitor the individual metabolic fingerprint of a biofluid or tissue and parallels the ability to identify clear signatures of quality in meat, which requires establishing a relationship between microbial growth and the chemical changes that occur during the storage period.[2] The "omics" approach can also identify the main nonvolatile compounds of meat deterioration.

The "omics" methodologies have been known for some time, and among these, the four largest are genomics, transcriptomics, proteomics, and metabolomics. Although most metabolomic studies use biological fluids or samples derived from living organisms, another approach has gained attention, which is the use of this knowledge in a wide variety of applications involving other types of complex samples, for example, food products.[5]

A variety of other omics approaches have emerged as diseasomics, metallomics, epigenomics, lipidomics, interactomics, and foodomics.[6] The latter seeks a holistic understanding of food analysis focusing on food safety and quality, and gives protection to consumers against adulteration. Though this research topic has grown considerably, the term foodomics was first used in 2009, by Cifuentes, who defined it as a "*discipline that studies the Food and Nutrition domains through the application of omics technologies.*"[7,8]

A Web of Science (Clarivate Analytics) search points to increasing interest in foodomics as observed in Figure 1, even overcoming the traditional methodologies like genomics and proteomics.

This new approach uses techniques established for other "omics" to investigate diverse and crucial issues in food science: food contaminants and toxicity; compounds related to quality and effects for human health; a profile of active compounds, authenticity, geographic characterization, physical, chemical, and microbiological effects of storage, etc.[9]

The most widely used technique to carry out foodomics[8] is mass spectrometry (MS), which has been identified as the most powerful analytical instrument for obtaining qualitative and quantitative information. However, other techniques have gained acceptance in foodomics, among them is nuclear magnetic resonance (NMR).[8]

Nuclear magnetic resonance is less sensitive than MS and requires greater sample quantities, while MS offers a better limit of detection (LOD) than NMR. However, NMR is a versatile, reproducible, nondestructive technique that can be applied to both liquid and solid matrices without derivatization and without reliance on ionizing steps or on column conditioning. NMR also allows for unambiguous metabolite assignment and offers several advantages over other analytical techniques such as high-pressure liquid chromatography (HPLC), mass spectrometry (MS) and gas chromatography (GC).[10]

Nuclear magnetic resonance has already been applied to study several food products namely: wine, beer, honey, vinegar, coffee, fruit juices, melon, tomato, potato, onion, watermelon, olive oil, milk, cheese, and butter.[11] Continued diverse applications of NMR in foodomics can be expected. For example, NMR is of utility to characterize time-dependent changes in stored beef, which blurs the lines between metabolomics and foodomics.

Recent work has studied the effects of different protective steps. The ^{1}H NMR metabolic profile of irradiated meat applying different doses has been evaluated, revealing that this technique was sensitive to metabolic differences caused by the irradiation.[12] Drying processes of meat extracts were monitored by ^{1}H NMR, revealing that eight among 32 metabolites were more abundant after moisture loss (drying process), including tryptophan, phenylalanine, valine, tyrosine, glutamate, isoleucine, and leucine.[13]

In addition, 48 meat samples of raw meat juice, exudate, and the whole piece of meat were compared using ^{1}H NMR and HRMAS (High-Resolution Magic Angle Spinning), for solid samples. Analyses of these spectra reveal that 95% of the peaks were coincident in both types of samples (solid and exudate) allowing the identification of 60 metabolites, through 1D and 2D NMR, of which 23 were reported.[14]

In this work, we examined the metabolic profile changes of *Longissimus dorsi* (Sirloin); *Psoas major* (Tenderloin) and *Trapezius toracis* (Chuck) with temperature and storage time, by ^{1}H NMR.

2 | MATERIALS AND METHODS

2.1 | Sample

Different type of cuts, such as *Trapezius toracis* (Chuck), *Longissimus dorsi* (Sirloin), and *Psoas major* (Tenderloin), were provided by a cattle slaughterhouse from São Paulo, Brazil. Meat cuts were obtained randomly, from the deboning line, and selected according to the final pH (pH range: 5.4-5.8). The pH was measured with the direct contact of the samples with the electrode (brand Testo 205, Brazil).

The pieces were sliced in steaks (~250 g) and vacuum-packed, with the support of the slaughterhouse operators

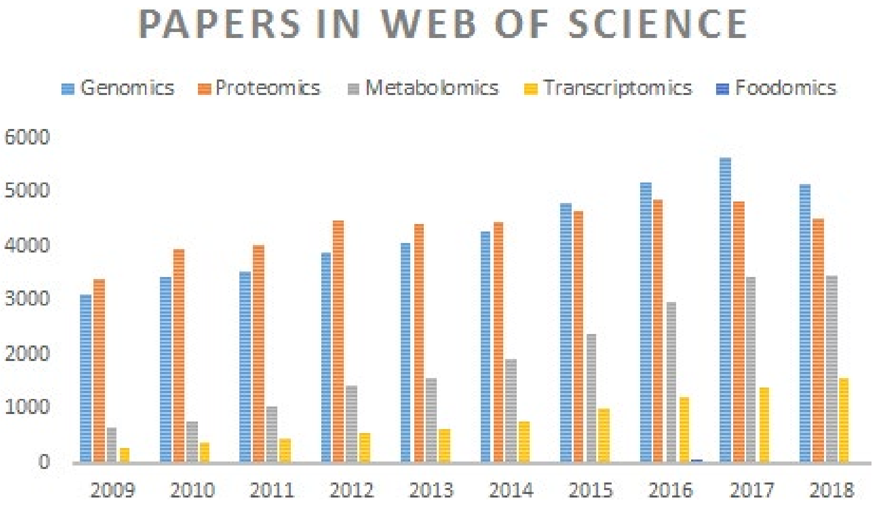

FIGURE 1 Comparisons of the numbers of "omics" publications in a 10-year window

TABLE 1 Samples of the three types of meat cuts with the respective storage temperatures

Conditions	Chuck			Sirloin			Tenderloin		
Temperature (°C)	0	4	7	0	4	7	0	4	7
Storage time (d)	0 and 49	0 and 28	0 and 28	0 and 49	0 and 28	0 and 28	0 and 49	0 and 28	0 and 28

and 24 hours later the samples were taken to the Laboratory of Quantitative Food Microbiology (FEA, UNICAMP, São Paulo, Brazil),. The transportation was in sealed Styrofoam boxes containing meat and ice in a 1:1 ratio. The samples were stored at 0, 4, and 7°C for subsequent analyses. The stored samples were retrieved after 0, 28, 35, and 49 days at 0°C; after 0, 10, 21, and 41 days at 4°C and after 0, 10, 22, and 42 days at 7°C.

In this study, the samples were collected immediately after the microbiological analysis. Meat samples (~5 g) were obtained, aseptically and randomly, from the meat surface, since this is the area that is mostly colonized by spoilage bacteria. Then, the samples were stored at −20°C.

A pilot experiment was conducted with the samples shown in Table 1 to confirm that the experimental methodology differentiated the samples in question, resulting in 12 analyzes, four for each meat cut.

It is worth mentioning that 0 is common to the three different temperatures of each cut. In this step, no experiment was performed in duplicate, since the objective here was to test a methodology that could differentiate the metabolites present at the different storage temperatures.

After confirming the viability of the extraction process, more samples were investigated, which are presented in Table 2. All samples were analyzed in duplicate, totaling 60 samples. Two samples showed contamination and were excluded from the analysis, S0D35(2) and S0D49(1).

2.2 | ^{1}H NMR samples preparation for the analyses

For the experiments, 250 mg of meat sample and 1.5 mL of solvent (D_2O) were used. Phosphate buffer (pH = 7.4, 50 mmol/L) was added to the buffered medium and the internal standard (1.0 mmol/L of TSP). Then, it was transferred to a microcentrifuge tube in which the solvent (D_2O) was added. The mixture was vortexed for 2 minutes and centrifuged at 13 000 rpm for 10 minutes at 4°C. After filtration of the supernatant, 0.5 mL of the sample was transferred to a 5 mm NMR tube.

2.3 | ^{1}H NMR spectroscopy

^{1}H NMR spectra were obtained using a Bruker Avance DRX-400 at 9.4 T (400 MHz ^{1}H frequency) equipped with an inverse detection probe (5 mm O.D.). The water signal was suppressed with a PRESAT pulse sequence.

All spectra were acquired without spinning at 25°C, with a spectral width of 4.8 kHz, 32 k digitalized points, accumulation of 32 transients and four "dummy scans," and with the acquisition time of 3.41 seconds. The interval between the pulses was set at 5 seconds, and the total time of experiments was set to 5 minutes. The spectra were processed in TopSpin 3.5 software. Before the Fourier transform, all the FIDs were subjected to apodization using an exponential function with line broadening equal to 1 Hz and zero-filling to 64k points.

Automatic phase and baseline corrections were applied and manually adjusted when necessary. The chemical shift of the spectrum was adjusted in relation to TSP ($\delta = 0.000$ ppm). The NMR peaks assignment and identification were performed by comparison with the literature data.[12,13,15]

2.4 | Multivariate analysis of ^{1}H NMR spectra

The multivariate analysis of the 58 meat extracts ^{1}H NMR spectra was conducted in MATLAB®. Spectra were not binned prior to statistical analysis. Alignment and normalization of these data with the *Icoshift* tool used an average spectrum as chemical shift reference.[16] The analysis included the regions between 0.50-4.55 and 5.20-9.00 ppm, excluding the water residual signal and others regions containing only noise. Pareto scaling pre-processing was applied to the data set (58 *samples* × 23.094 *points*) and PCA was performed to mean-centering data.

TABLE 2 Samples of the three meat cuts with the respective storage temperatures

Conditions	Chuck			Sirloin			Tenderloin		
Temperature (°C)	0	4	7	0	4	7	0	4	7
Storage time (d)	0, 28, 35, 49	0, 10, 21, 41	0, 10, 22, 42	0, 28, 35, 49	0, 10, 21, 41	0, 10, 22, 42	0, 28, 35, 49	0, 10, 21, 41	0, 10, 22, 42

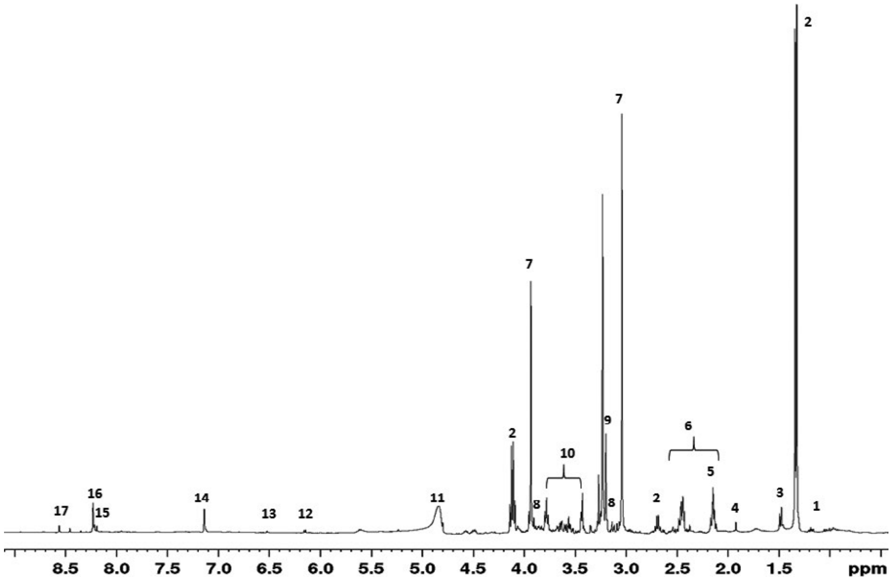

FIGURE 2 A representative ^{1}H NMR spectrum of Chuck extract, day 0. Legends: (1) leucine/isoleucine/valine; (2) lactate; (3) alanine; (4) acetate; (5) methionine; (6) glutamine/glutamate; (7) creatine; (8) creatinine; (9) betaine; (10) glycerol/glicine/carbohydrates region; (11) residual water; (12) anomeric hydrogen of ATP/ADP/hypoxanthine/inosine; (13) fumarate; (14) carnosine; (15) adenine/hypoxanthine; (16) adenosine/inosine/carnosine; (17) ATP

3 | RESULTS AND DISCUSSION

In metabolomics, two routes are possible: the individual study of each metabolite characterized in the ^{1}H NMR spectrum (targeted), or the analysis of the total spectrum profile (untargeted). In targeted analysis, the metabolites are identified and quantified, and the metabolite concentrations are analyzed by multivariate techniques followed by the chemical/biological interpretation. In the second route, a reverse approach is applied: the total spectrum is analyzed by multivariate tools to determine the ability to classify samples; this approach employs graphical formats that aid in identifying the most important spectral regions and metabolites that allow the discrimination of the samples.

The focus of present work will be the untargeted approach, in which the total spectrum is analyzed and then the results of the multivariate analysis will be compared to assigned peaks for numerous metabolites including valine, leucine, isoleucine, lactate, lactate, alanine, glutamine, methionine, acetate, creatine, creatinine, betaine, glycerol, glycine, fumarate, carnosine, adenosine, adenine, and ADP among others (Figure 2).

A pilot analysis was conducted first on 12 samples (three cuts of meat at different storage condition), where the PCA model explained 50.6% of the data variance with two principal components (Figure 3). The score plots show a separation of samples along the first principal component associated with increasing storage temperature. In this sense, the ^{1}H NMR spectra from meat extract can be used to evaluate the meat decomposition.

The preliminary results suggested meaningful trends and therefore the methodology was applied to a larger data set (58 samples described in Table 2). The PCA model of the ^{1}H NMR data with two principal components explained 47.4% of data variance in this larger sample set. The score plots show a separation of samples along with the storage, reflecting possible meat degradation (Figure 4).

For clarification, the samples were denominated taking into consideration the type of cut, the temperature of storage, and how much time was stored. For example, T4D41(2)—Tenderloin, 4°C, stored for 41 days, sample 2; C0D28(1) Chuck, 0°C, stored for 28 days, sample 1; S7D22(1)—Sirloin, 7°C, stored for 22 days, sample 1.

Samples in nature (time zero) were associated with negative PC1 and PC2 scores. Longer meat storage time led to progressively more positive PC1 and PC2 scores. Therefore, the bisector of the odd quadrants can be associated with a temperature degradation of meat/or extended storage times. Kodani et al[17] demonstrated that ^{1}H NMR profiles of water-soluble metabolites revealed overall metabolic change linearly correlated with beef aging rate and aging duration.

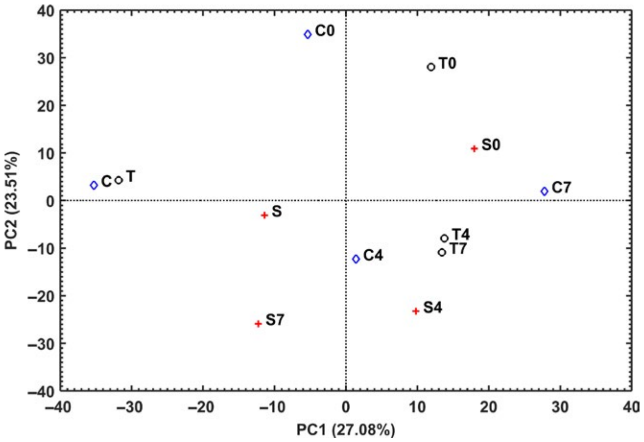

FIGURE 3 Score plot from the PCA model of the 12 meat samples. Legend: (o) Sirloin-S; (+) Tenderloin-T; (◇) Chuck-C; (0) 0°C; (4) 4°C; (7) 7°C. The storage times are shown in Table 1

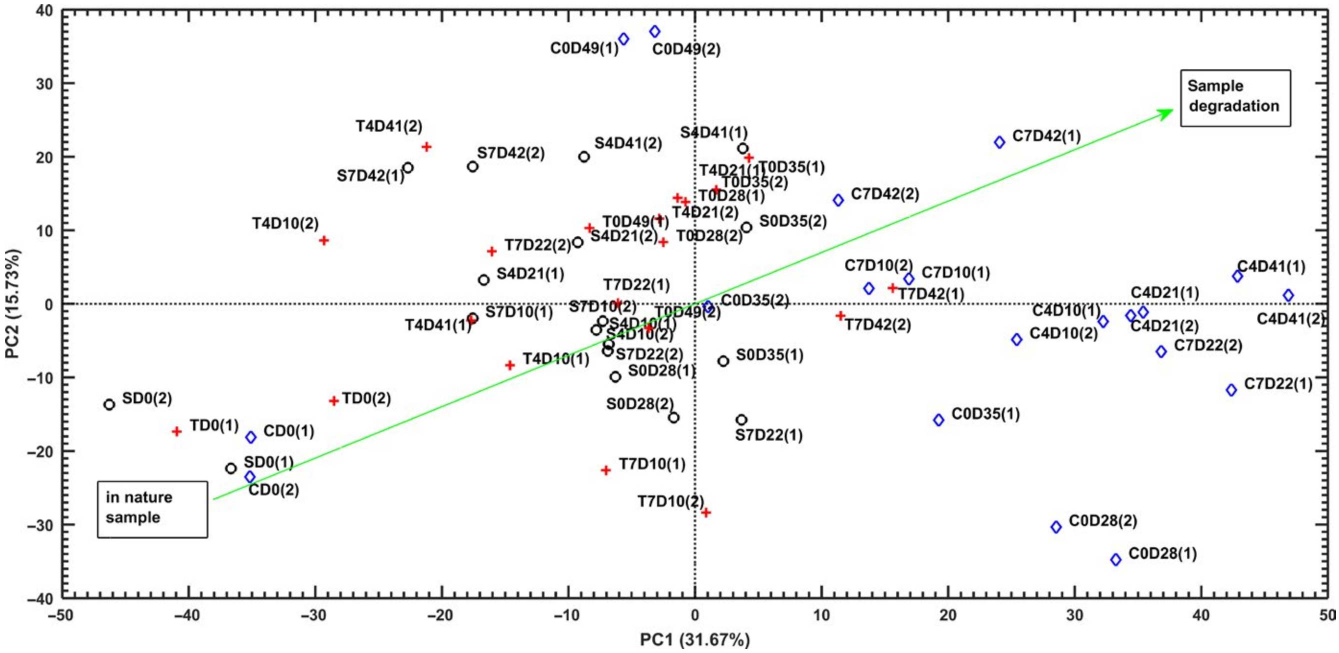

FIGURE 4 Score plot from the PCA model of the 58 meat samples. Legend: (o) Sirloin; (+) Tenderloin; (◊) Chuck. (0) 0°C; (4) 4°C; (7) 7°C. Meaning of the captions, for example: T4D41(2)—Tenderloin, 4°C, stored for 41 days, sample 2; C0D28(1) Chuck, 0°C, stored for 28 days, sample 1; S7D22(1)—Sirloin, 7°C, stored for 22 days, sample 1. The storage times are shown in Table 2

Chuck samples are seen to be more sensitive to storage degradation, showing significant metabolic alterations and shifting toward higher positive scores in PC1, whereas the majority of Tenderloin samples generally exhibited negative PC1 scores, an indication of a lower degradation tendency. Finally, the score plot shows the tendency of separation between Sirloin, Tenderloin, and Chuck along the first principal component.

The score plot also highlights the importance of storage temperature on the timescale of degradation and possible growth of the microbial populations. For example, samples of Tenderloin stored for 10 days at 7°C (T7D10) showed similar degradation to samples stored at 4°C for 22 days (T4D22) or samples stored for 49 days at 0°C (T0D49).

The plot of the PCA model loadings (Figure 5) showed the variables (chemical shifts) that most influenced the grouping of samples observed in the score plots (Figure 3). These chemical shifts can be assigned to chemical compounds present in the meat extract and correlated with the metabolic functions (Figure 5).

The loading plots highlighted nine chemical markers candidates to evaluate the meat storage degradation, and higher relative abundance of leucine, isoleucine, valine, alanine, and acetate were found in samples stored at high temperatures or for long times. This observation was taken as indicative of amino acid production by proteolytic degradation.[18]

Rabie et al[19] achieved similar results by monitoring the concentration of free amino acids and biogenic amines for

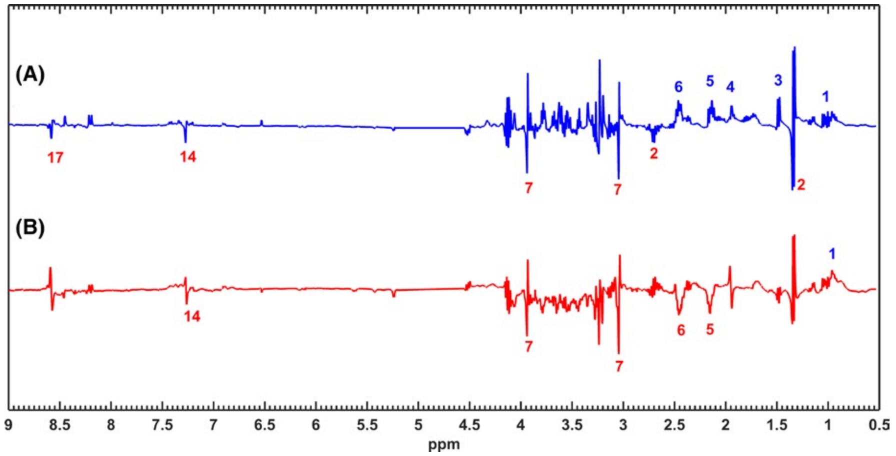

FIGURE 5 Loadings plot of PCA model 58 meat samples. A, first principal component (31.67%), and second principal component (15.73%). Legends: (1) Leucine/isoleucine/valine (increase); (2) lactate (decrease); (3) alanine (increase); (4) acetate (increase); (5) methionine (positive in PC1 and negative in PC2); (6) glutamine/glutamate (positive in PC1 and negative in PC2); (7) creatine (decrease); (14) carnosine (decrease) and (17) ATP (decrease)

samples stored at 4°C for 28 days, revealing that their presence increased with increasing storage time when investigating horse, beef, and turkey sausages. Nishimura et al[20] also found a metabolite increase of alanine, taurine, leucine, serine, valine and glutamine during storage.

The relative abundance of lactate, creatine, carnosine, and ATP decreased with the sample storage. A possible explanation for this trend may be the decomposition of compounds such as hydrolysis of the dipeptide carnosine to alanine and histidine, supported by the observed increase in alanine content.[21]

The degradation of ATP/ADP during storage is well documented and may generate the following metabolites: AMP, IMP, inosine, hypoxanthine, xanthine, and uric acid.[22] A similar decrease in the lactate concentration was observed by Nishimura et al[20] this decrease could be associated with bacterial degradation.[2]

Methionine, glutamine, and glutamate have interesting behavior. These compounds have a positive contribution to PC1 and a negative contribution to PC2. Comparing both sides of the bisector of the odd quadrants, we can observe that Chuck samples were with positive values in PC1 and negative values in PC2. In contrast, Sirloin and Tenderloin samples show the opposite trend, suggesting that methionine, glutamine, and glutamate may be more sensitive to meat classification than degradation.

4 | CONCLUSION

Different processes such as proteolysis of muscle proteins, microbial growth, and lipid oxidation coincide during storage of butchered, packaged meats, and ^{1}H NMR is an analytical technique capable of monitoring these events. Moreover, the multivariate analysis of NMR spectra of aqueous meat extracts can be considered an excellent ally to characterize the composition and metabolic profile of the meat during storage at different temperatures and the degradation extent. This methodology may also be sensitive to the influence of the meat cuts on the growth of mixed microbial populations. Higher relative abundance was observed for leucine, isoleucine, valine, alanine, and acetate in samples stored at high temperatures or for long times, while the relative abundance of lactate, creatine, carnosine, and ATP was lower in samples withstanding more extended storage.

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