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Metabolomics of meat exudate: Its potential to evaluate beef meat conservation and aging

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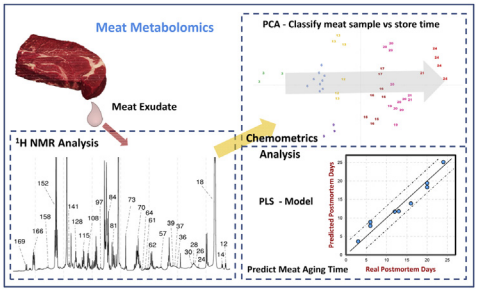
HIGHLIGHTS

- NMR spectra from beef samples and their exudates are very strongly correlated.
- 23 metabolites not reported in previous NMR meat studies have been identified.
- Meat exudate NMR spectra allow monitoring of biochemical changes related to aging.
- PCA of exudate NMR spectra classified meat samples by their storage time.
- The aging of a meat sample can be predicted by PLS analysis of its exudate.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study we analyzed the exudate of beef to evaluate its potential as non invasive sampling for nuclear magnetic resonance (NMR) based metabolomic analysis of meat samples. Exudate, as the natural juice from raw meat, is an easy to obtain matrix that it is usually collected in small amounts in commercial meat packages. Although meat exudate could provide complete and homogeneous metabolic information about the whole meat piece, this sample has been poorly studied. Exudates from 48 beef samples of different breeds, cattle and storage times have been studied by ^{1}H NMR spectroscopy. The liquid exudate spectra were compared with those obtained by High Resolution Magic Angle Spinning (HRMAS) of the original meat pieces. The close correlation found between both spectra (>95% of coincident peaks in both registers; Spearman correlation coefficient = 0.945) lead us to propose the exudate as an excellent alternative analytical matrix with a view to apply meat metabolomics. 60 metabolites could be identified through the analysis of mono and bidimensional exudate spectra, 23 of them for the first time in NMR meat studies. The application of chemometric tools to analyze exudate dataset has revealed significant metabolite variations associated with meat aging. Hence, NMR based metabolomics

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have made it possible both to classify meat samples according to their storage time through Principal Component Analysis (PCA), and to predict that storage time through Partial Least Squares (PLS) regression.

1. Introduction

Beef is one of the meat products with increased demand and commercial value. In the western world its consumption has markedly grown in the last few decades [1]. As a consequence, there is an increasing need for proper analytical methods capable of giving a complete picture of muscle metabolome and of assessing meat nutritional quality [1,2]. One of the main aspects that contributes to the sensory quality of beef is its flavor, which is directly related to the chemical composition, especially of such low molecular weight compounds as nucleotides, amino acids, dipeptides and sugars [3], which have been related to meat aging processes [3,4]. The levels of these compounds in beef meat are usually determined by extraction procedures followed by analysis, which often imply the use of different analytical techniques for each group of metabolites. For example, Koutsidis et al. [5] used Gas Chromatography Mass Spectroscopy (GC–MS) to measure sugars, capillary electrophoresis (CE) to characterize the nucleotides and the derivatization technique EZ-Faast (Phenomenex, Torrance, CA) coupled with GS-MS to analyze amino acids.

High resolution Nuclear Magnetic Resonance (NMR) spectroscopy is a well established technique that, when applied to complex mixtures, allows the simultaneous detection of a large number of their low molecular weight components. Hence, NMR spectroscopy is gaining wide acceptance in food analysis due to its potential for giving very exhaustive representations of the chemical composition of the food matrix without extensive manipulation [2]. Particularly, NMR spectroscopy has been employed to obtain metabolite profiles of meat samples, including different cattle breeds [6], beef [1,3,7,8], duck [4], horse [9], pig [10] or lamb [11]. For this goal, the NMR spectroscopy modality known as High Resolution Magic Angle Spinning (HRMAS-NMR) has proved to be very useful for reliably assessing the metabolic profile (metabolome) of intact muscle samples [6,7,11]. Thus, this modality has been applied to investigate factors that somehow affect meat properties, such as the geographical origin of cattle [1,7,11], type of cattle feeding [11], type of breeds [6] or the relationship between meat aging and flavor [3,4,12]. Nevertheless, in comparison with other foods, meat has hardly been investigated by NMR [6].

Meat exudate is the aqueous solution, mainly constituted by proteins and their degradation byproducts, which comes out of fresh meat during storage. Different theories have been proposed to explain the physicochemical mechanisms involved in meat exudation or drip loss, the most accepted being shrinkage of myofibrils during rigor development [13], and occurrence of tissue damage due to cutting [14]. However, the reasons why drip losses vary from carcass to carcass, even under controlled environmental conditions, are not fully understood [15].

Meat exudates mainly contain water soluble sarcoplasmic proteins mixed with nucleotides, amino acids, peptides, proteins, and many soluble enzymes [16]. They are valueless, easy to obtain and, their presence in small amounts in commercial meat packages cannot be associated with significant degradation or alteration of the meat from which they proceed. They should, therefore, be considered as the natural juice from raw meat that, conveniently analyzed, could provide homogeneous information about the whole meat sample. Hence, the composition of pork exudates has been correlated with quality characteristics of fresh and thawed meat [17], and with water holding capacity and postmortem aging [18]. However, in spite of this potential, meat exudates have rarely been employed for meat analysis.

Therefore, the aims of this study were i) to assess the value of beef exudates for NMR based meat analysis, ii) to characterize the major metabolites present in beef exudates, iii) to evaluate the ability of ^{1}H NMR to characterize the chemical changes in beef exudate during storage and iv) to apply NMR based chemometric techniques to classify beef samples according to postmortem time (aging).

2. Materials and methods

2.1. Sample collection

Forty eight raw beef tenderloins (psoas major, psoas minor and iliacus) were collected from five local butcher's shops. Meat samples differed in their postmortem periods, which ranged from 3 to 24 days [3 days postmortem (3 samples), 6 days (9), 9 days (3), 12 days (3), 13 days (4), 16 days (6), 17 days (3), 19 days (3), 20 days (4), 21 days (6), and 24 days (4)]. According to local regulations, meat had been stored before purchasing at 4 ± 1 °C with a relative humidity of 83–85%. Thermally insulated containers were employed during transportation of the samples to the laboratory, which, in all cases, took no longer than 1 h. Once at the lab, samples were kept refrigerated at 4 °C until being subjected to the following exudate collection procedure: the same day of purchase, 50 g pieces of beef samples were vacuum packed at 20 kPa in 10 × 10 cm laminated film bags (90 μm copolymer of polyamide/polyethylene) of low gas permeability (transmission rates of 35 cm^3 24 h^{-1} m^2 bar^{-1} and 150 cm^3 24 h^{-1} m^{-2} bar^{-1} for O_2 and CO_2, respectively) and stored at 4 °C. After 24 h storage the released exudate was collected, frozen at −80 °C, and freeze dried at room temperature. The freeze dried exudates were stored at −80 °C until NMR analysis. Six representative samples of beef tenderloins, after collecting their exudates, were frozen at −80 °C until HRMAS-NMR analysis.

2.2. Preparation of samples for NMR analysis

For ^{1}H NMR analysis 18 mg from each lyophilized exudate were reconstituted in an eppendorf tube by adding 650 μL of D_2O, containing 1 mM sodium trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP, SigmaAldrich) as internal reference. The mixture was vortexed for 30 s and then transferred to a 5 mm NMR tube.

For ^{1}H HRMAS analysis, 10 mg samples of beef muscle together with 25 μl of 1 mM TSP were placed into 50 μl MAS zirconia rotors and fitted with top cylindrical inserts to increase homogeneity.

2.3. NMR analysis

2.3.1. One dimensional NMR experiments

^{1}H NMR spectra of the reconstituted exudates were recorded randomly at 277 K on a Bruker AMX500 MHz spectrometer using a reverse detection probe (Bruker BioSpin GmbH, Rheinstetten,