Effect of storage of dry-aged beef under vacuum by 1H NMR spectroscopy

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Objectives: In this study, the effect of storage in vacuum packaging of dry-aged beef after its aging on the polar fraction of metabolome was investigated by ^{1}H NMR spectroscopy. The storage time in vacuum packaging varied between 2 and 14 days.

Materials and Methods: Raw material: Right bone-in strip loins of six Simmental young bulls. Aging method: Right strip loins were dry-aged in a dry aging chamber. Aging parameters: Aging time was varied between 0, 7, 14, 21 and 28 days for each animal. Sam- ple preparation after aging: The crust of dry-aged samples was trimmed, followed by cutting the piece in half and removing one slice for NMR measurement and a second slice that has been stored under vacuum for 2, 5, 7, 9, 12 or 14 days. Sample preparation for measurement: 0.2 g of sample was homogenized with 0.4 mL methanol, 0.2875 mL Millipore water and 0.4 mL chloroform, followed by a 10 min cooling on ice. The samples were centrifuged at 6900 rpm (20 min, 4 °C). The upper phase was dried under vacuum. Dissolving the extract in 200 mM Na_2HPO_4 + 10 % D_2O + 0.001 % NaN_3 + 80 µL internal standard (100 mM imidazole + 11 mM maleic acid + 0.33 % TSP-d4). After adjusting the pH to 7, 600 µL are filled in a 5 mm NMR tube. Measurement: A Bruker 400 MHz Ascend NMR spectrometer was used for NMR analysis. ^{1}H-NMR spectra were acquired for each sample with a noesygp- pr1d pulse sequence with following parameters: spectral width, 8.403 kHz; number of points, 65 k; number of scans, 128; number of dummy scans, 4; acquisition time, 3.9 s; presaturation field strength for water suppression, 25 Hz. The processing of the NMR spectra was performed by Topspin 3.5 pI 7 (Bruker Biospin GmbH). Metabolite identification: To identify the metabolites in ^{1}H- NMR spectra, a comparison with NMR database and literature was performed. Standards for different metabolites were measured using the same method to substantiate the comparison of sample and database. Data analysis: The Data were analyzed with the software Matlab R2018a. The statistical analysis to determine aging type was based on principal component analysis (PCA). To analyze correlations between metabolite content and aging time, the correlation coefficient with alpha of 0.05 was used.

Results and Discussion: The storage in vacuum packaging of dry-aged beef samples after aging had an effect on the polar fraction of metabolome. The PCA of dry-aged samples showed a shift for aging time from left to right. It was found that the samples stored under vacuum after dry-aging had an additional shift with higher principal component 1 values than the non-stored dry-aged samples. This indicates that the metabolome is affected by the additional storage time under vacuum. The levels of metabolites leucine, isoleucine, and valine correlated positively with the storage time under vacuum after dry-aging, whereas the levels of IMP showed negative correlations. The correlation coefficients of lactic acid, glutamate, inosine and acetic acid differed according to the dry-ag- ing time.

Conclusion: Storage dry-aged beef in vacuum packaging after dry-aging affects the metabolome of beef, and therefore possibly the aging outcome and the taste of beef.

Key words: Dry-aging, Storage, Beef, Vacuum, Metabolome